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KALA-AZAR



Plate No I.

An Indian boy suffering from Kala-Azar; untreated.

KALA-AZAR

A handbook for Students and Practitioners

by

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TO

MY COLLEAGUES

AT THE

CALCUTTA SCHOOL OF TROPICAL MEDICINE

PREFACE

In 1918 Dr E. Muir published a small book on the diagnosis and treatment of kala-azar. This book, written by one of the pioneers in the treatment of the disease, met a long-felt need, and in a year or so the whole edition was sold out. Dr. Muir, who was at this time engaged on leprosy research, suggested to me that I should collaborate with him and re-write the book. In 1923 Dr. Muir and I published a book, which, although considerably enlarged, was virtually the second edition of his original small handbook.

This second book is now not only out of print, but is entirely out of date. During the last few years an immense amount of work has been done on the transmission problem, and great advances have been made in the treatment of the disease. Dr. Muir has been engaged exclusively on leprosy research work for a number of years, so that it has fallen to my lot to write the present volume.

I have been able to collect a number of additional facts with reference to the epidemiology of the disease, so that the chapter on this subject is longer and, it is hoped, more accurate.

For the last two and a half years, since my colleagues and I at the Calcutta School of Tropical Medicine first incriminated the sandfly, *Phlebotomus argentipes*, work on the transmission problem has been concentrated on this insect; the results of almost all the recent work done on this problem in India suggest that this sandfly is the transmitter, but actual transmission by its agency has not yet been effected. As the transmission problem is thus unsolved, I have discussed it in some detail—not with the object of establishing any particular hypothesis, but in order to place the whole problem, as it now stands, before the reader, who, if he is engaged in practical work in the same field, may be in a position to supply facts and, thereby, to add to our general knowledge of the subject.

The chapter on prophylaxis is a short one. If another edition of this book appears, I hope that I shall be able to add to this chapter at the expense of the chapter on ætiology—much of which will be entirely superfluous directly the transmission problem is solved.

With the introduction of the pentavalent compounds of antimony the whole problem of treatment has undergone a great change. Three years ago a cure rate of about 80 per cent. was the

best that could be anticipated in a mixed population; now a 95 per cent. cure rate is well within reasonable expectation. The minimum course of treatment, which previously extended over a period of two months, now occupies one quarter of that period, and a lower relapse rate can be anticipated after eight injections of at least one of the pentavalent compounds than was the rule after thirty injections of sodium antimony tartrate. Certain patients who 'resisted' treatment by the antimony tartrates can now be cured with comparative ease by the substitution of a pentavalent compound. But here again finality has not yet been reached, and there are still a few patients who are not cured by any of the antimony compounds at present in use.

These advances in the treatment of the disease have necessitated the complete re-writing of the chapter on this subject; and, as the comparatively high cost of the pentavalent compounds stands in the way of their universal adoption, I have thought it advisable to repeat the details of treatment by the antimony tartrates, and, at the same time, to give the further details necessary for using the pentavalent compounds.

A separate chapter has been devoted to the subject of morbid anatomy and histopathology, and this subject has been dealt with in a little more detail.

The chapters on symptomatology, laboratory methods and diagnosis have been re-written, but are essentially the same as in the previous book.

I am greatly indebted to all my colleagues at the Calcutta School of Tropical Medicine for their generous and invaluable help. My thanks are especially due to Lieut.-Col. H. W. Acton, I.M.S., Professor of Pathology, for assistance with histological material and with the chapter on this subject; to Major R. Knowles, I.M.S., for permission to use his coloured illustrations, plates nos. xii and xiii; and to Dr. J. M. Henderson, for assistance in reading and correcting the proofs.

My thanks are also due to the Editor of the *Indian Journal of Medical Research*, for allowing me to use a number of blocks which are the property of this *Journal*; and to the Director of the Calcutta School of Tropical Medicine, for the use of the colour blocks, from which plate no. ix is printed.

Calcutta, L. EVERARD NAPIER.
April 27, 1927.

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DEFINITION

KALA-AZAR, or black fever, is an irregular fever of long duration, with either a rapid or a slow onset, occurring epidemically and endemically in certain tropical and sub-tropical countries, usually associated with progressive emaciation and enlargement of spleen and liver and characterised by the presence of a parasitic protozoon, *Leishmania donovani*, in the peripheral blood, in the spleen, and elsewhere in the tissues.

GEOGRAPHICAL DISTRIBUTION

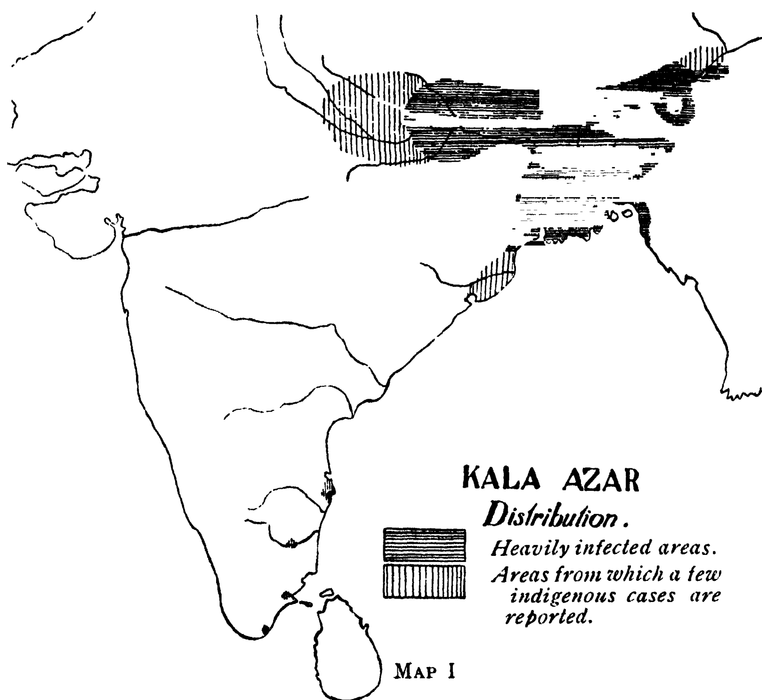
IN INDIA

The most heavily infected areas are in Bengal, Bihar and Assam. There are areas of a lower degree of endemicity in the United Provinces, Madras and Orissa. Map I gives a general idea of the distribution in India.

Bengal.—Kala-azar is endemic in every district of Bengal. The report of the Director of Public Health, Bengal, for 1925, which shows that 179,041 patients were treated during the year, gives some idea of the number of cases that there are in the province. Although no special organisation for the treatment of kala-azar that is any way comparable with that in operation in Assam has yet been established in Bengal, a considerable amount of interest has been centred around the disease during the last few years. In the out-patient department of the Calcutta School of Tropical Medicine alone we treat more than a thousand patients a year. The patients come from all over Bengal, and the number

from each district usually varies in inverse ratio to the distance of the particular district from Calcutta.

The dispensary returns do not give an accurate idea of the relative incidence of the disease in the various districts; this is shown in the dispensary returns for Howrah district, which were given as 20 for the year 1922, whereas in our out-patient department we treated about 80 cases from this district during that year. However, both the dispensary returns and our own experience



MAP I

suggest that there are relatively few cases in Midnapore district, and even fewer in Bankura and Birbhum. In the last named districts the most recent returns show that less than 0·1 per cent. of the total dispensary attendances are of patients suffering from kala-azar; whereas in some districts kala-azar cases constitute 2·6 per cent. of the total attendances. The numbers of kala-azar cases treated in the dispensaries of these three districts during 1924 are, respectively, 193, 37 and 79.

Bihar and Orissa.—In this province the distribution is very interesting. The districts which lie in the Indo-Gangetic plain seem to be very heavily infected, but the districts on the low laterite hills seem to be entirely free from indigenous cases of the disease. The dispensary returns show that in Muzaffarpur 3,235 cases of kala-azar were treated during 1921 as against 16 cases in Ranchi. From the coastal part of Orissa few cases are reported. We have seen a few definitely indigenous cases from both Cuttack



KEY MAP

and Balasore, but it is quite obvious that these are areas of low endemicity as compared with the districts in the north of the province.

Assam.—The whole of the Brahmaputra valley, with the exception of the Jorhat sub-division of the Sibsagar district and the Dibrugarh sub-division of the Lakhimpore district, is heavily infected. The Garo Hills district is also infected. In a recent

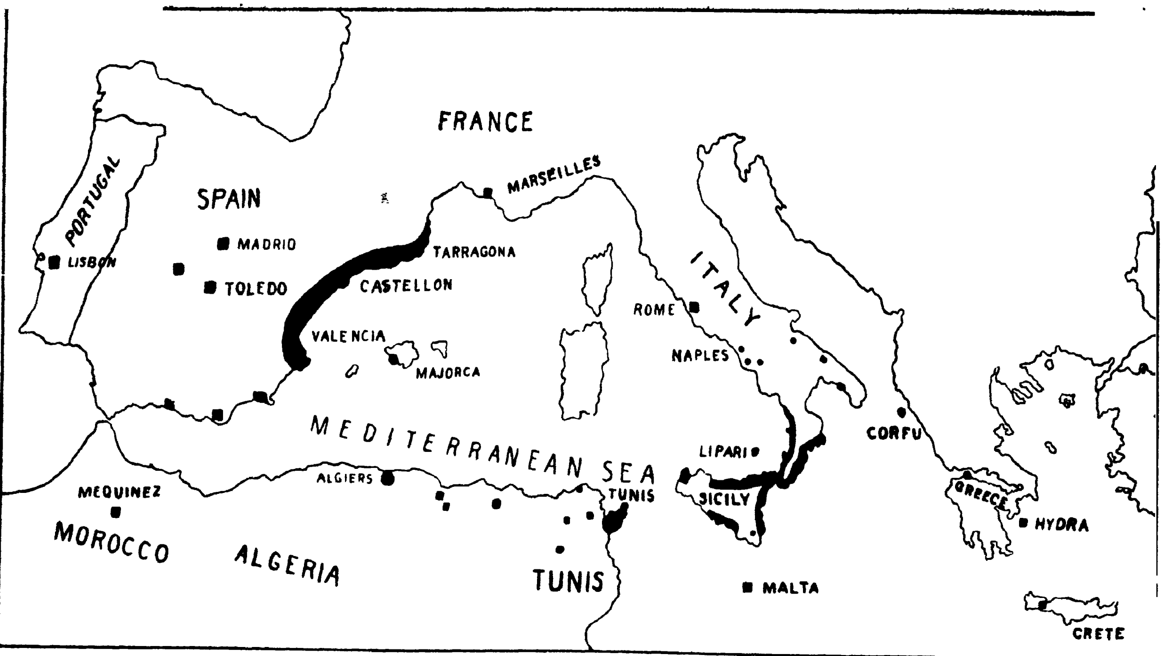
report the Director of Public Health for Assam remarks that the whole of this district seems to be infected, but that 'it is more infected in the lower-lying, water-logged land on its boundaries tha in the hilly interior'. The Khasia and Jaintia Hills are apparently free from infection, as also are the Naga, and higher parts of the Cachar Hills. The disease seems to be endemic all over Sylhet, and recently a number of infected villages have been found in Cachar. The medical officers of the Assam-Bengal Railway are very familiar with the disease in the plains' portion of the railway, but they find that it is rare in all portions of the hill section—which is the link between Sylhet and the Brahmaputra valley—and absent from the villages above 2,000 feet.

Madras.—Practically all the cases reported in the province have been from Madras City itself (Cunningham and Varadarajan, 1923); a few odd indigenous cases have been reported from Ponneri, Trichinopoly and Ramnad; and quite recently a new epidemic focus has been found in the extreme south of the province, only 50 miles from Cape Comorin (Cunningham and Pundit, 1925).

United Provinces.—Just over the border line which separates this province from Bihar the locality is fairly heavily infected; but as one goes west fewer and fewer cases are encountered, and it has been repeatedly stated by careful observers that cases do not occur west of Lucknow.

OUTSIDE INDIA

The disease has been known to exist in China for a number of years. Cochrane (1913) made a study of the distribution of the disease, and more recently Young (1923) collected the available information on this subject. It is evident that the distribution is a wide one. The largest number of cases is reported from the provinces on the coast north of the Yangtse river, Chih-li, Shantung, Kiangsu and Anhui being heavily infected. The information from the other provinces north of the river appears to be much more scanty, but here and there centres of infection are reported. South of the Yangtse river there is, however, no evidence that the disease occurs; a number of cases resembling kala-azar clinically have been reported, but microscopically proven cases are few and there is little evidence as to the local origin of these cases.



MAP II

The distribution of kala-azar in the Mediterranean area. The endemic areas are marked in black.

There are areas in Russian Turkestan where the disease is endemic.

In 1910 an epidemic in the Sudan was investigated by Bousfield, Thomson, Marshall and others (1911). Reports suggest that this epidemic was more closely related to the Indian than to the Mediterranean type of the disease.

Mediterranean, or infantile, kala-azar is similar in every way to the Indian variety, except that it attacks infants and young children almost exclusively. The coastal areas of Italy and Sicily have provided the largest number of cases of this type of the disease, but practically the whole of the Mediterranean littoral is an endemic area.

An investigation recently carried out in Spain by Pittaluga (1925) showed that the provinces on the east coast, especially Tarragona, Castellon and Valencia, are most heavily infected. There is also a focus on the south coast, another on the west coast of Portugal, and one in the centre of the peninsula in the provinces of Toledo and Madrid.

A few cases have been reported from the south of France; Malta and certain Greek islands are well-recognised endemic areas; Palestine and Egypt are apparently free from the disease; but cases are reported from Algiers and Tunis (see Map II).

Identification of Kala-azar as a Disease Entity

Kala-azar was not recognised as a definite clinical syndrome, or at least was not described as such in medical literature, until 1882, when Clark described the epidemic that had been raging in Assam for some years prior to this date. The reports of the Sanitary Commissioners of Bengal about this time suggest that the disease was looked upon as a separate clinical entity; later, however, after the discovery of the malarial parasite by Laveran in 1881, the fact that these parasites were frequently found in the blood of kala-azar patients led to the subsequent confusion between the two diseases.

During the two decades between the first clinical description of the disease and the discovery of the Leishman-Donovan body, there was a considerable difference of opinion about the nature of this

disease. In 1890 Dr. Giles, who was investigating the epidemic in Assam, described it as 'undoubtedly ankylostomiasis'. Rogers (1897), and later Ross (1899), reporting on the same epidemic, both gave their opinion that it was a form of malaria; whereas Bentley (1902), who was strongly opposed to the malaria theory, was misled by agglutination results with *Micrococcus melitensis* and suggested that it might be Malta fever. There were of course other observers, including the late Sir P. Manson and Col. Stephen (1897), who insisted on the distinctive nature of the disease, but this difference of opinion amongst some of our most prominent experts on tropical diseases is sufficient to show that even the epidemic form of the disease was not very distinctive, and it is easy to understand how the sporadic occurrence of the disease, as such, was overlooked.

THE HISTORY OF KALA-AZAR IN INDIA

The history of the disease prior to 1875 is by no means clear. It undoubtedly existed in Bengal before this date. Whether the disease which, making its appearance in Jessore district, swept across Bengal about the middle of last century was the début of kala-azar in the province, or whether it was a recrudescence of the disease in an already endemic area, is not known and will always be a matter of controversy. The latter view is supported by the fact that there is fairly definite evidence of the existence in Bengal of a fever which had many of the features of kala-azar, as early as 1835 (Twining), and that, although there is no record of this epidemic having spread through certain districts in Bengal, as, for example, Mymensingh, yet there is evidence that the disease has existed in these districts in sporadic form for a number of years. It is, however, quite certain that the disease which was described by Dr. French in 1872 as having existed in the Burdwan district since 1862 was kala-azar. The disease which attracted a considerable amount of attention in Rangpur and Dinajpur in 1872, in Bihar in 1882, and in other parts of Bengal about this time, was also undoubtedly the same disease, and was recognised as such at the time.

When the disease first made its appearence in Bihar it was confined to the alluvial plains, and it was hoped by the authorities at that time that its spread would be checked by the laterite hills

in the district. As the epidemic wave swept on, it did not at first appear to confine itself to the alluvial plain, but spread on to the adjoining laterite soil. It did not, however, establish itself on this soil, but rolled back upon the alluvial plain, which is the only part of Bihar where it is now endemic.

There is considerable evidence to show that the disease first made its appearance in Assam about the year 1875. The disease is reputed to have started in the Garo Hills, and to have spread from thence up the Assam valley, at first on the south of the river, and then through the northern portions of the valley.

It is rather interesting that a disease which has subsequently confined itself almost entirely to the plains should have started in the hills. At whatever point one takes up the story of the disease in the Garo Hills, reference is made to the previous existence of the disease there, but the writer usually adds that at the time of writing the disease has practically ceased to exist in the hills and is confined to the villages at the foot of the hills. There does not seem to be a clear account of an epidemic focus having existed at any time in the higher portions of the district. The population of the Garo Hills district has shown a steady increase from the year 1872 up to the present time, whereas many districts in Assam have shown periods of decrease—for which the ravages of the disease have been held responsible. The disease was at its worst in Nowgong between the years 1890 and 1900; and during this decade the population showed a 25 per cent. decrease.

For many years the disease did not spread beyond the Golaghat sub-division of the Sibsagar district, but in 1917 an endemic focus was found in the Sibsagar sub-division of this district, although the intervening Jorhat sub-division had escaped infection. The last reports show that a number of indigenous cases have been discovered in North Lakhimpur, but apparently the Dibrugarh sub-division is still comparatively free from the disease.

Rogers, who considers that the Assam epidemic was a direct extension of the Rungpur epidemic of 1872, describes the disease as having maintained a steady rate of progress up the valley, which rate he estimates at about ten miles a year. There is a tendency nowadays amongst investigators to discredit this theory of the invasion of Assam by the disease at this time; they seem to think that the disease existed in all parts of the province many years prior to 1875.

The fact that the disease has appeared from time to time within comparatively recent years in epidemic form in Assam, whereas in Bengal it has existed in endemic form only for many years, suggests that in the former province virgin soil was being invaded.

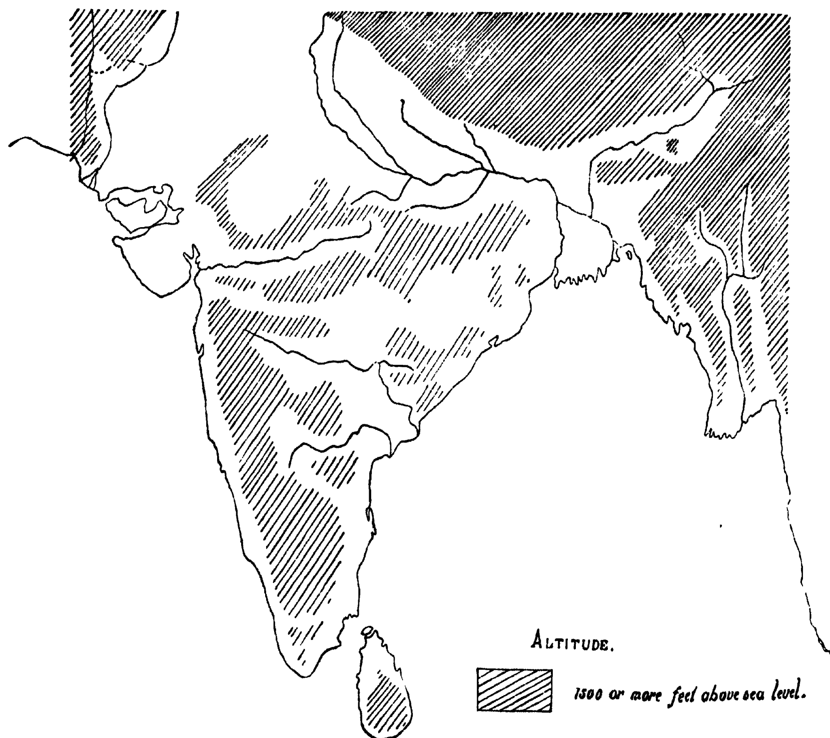
Limits of Penetration

Whether the disease has or has not spread from Bengal into the Assam valley within recent years, there seems to be little doubt that there has been no recent extension of range in any other direction. Communications radiate freely in a number of directions from the highly-kala-azar-infected areas in Bengal and Bihar. It is obvious, if one considers the number of infected persons there are in the endemic areas, that a very large number must from time to time leave these areas; the habits of the populations of the adjoining areas are not very markedly different, but nevertheless beyond certain definite limits the disease does not appear to spread. The extension of the disease appears to be limited on the east by the range of mountains that divides Bengal from Burma; in a north-easterly direction it has spread into Assam, and it seems possible that the extension up this valley is still continuing; the northerly extension is checked by the Himalayas; in a north-westerly direction it has spread into the United Provinces, but the further extension here appears to be checked by the entirely different climatic conditions which are encountered; the westerly extension appears to be checked by the laterite hills of Bihar; in a south-westerly direction it has extended a short way into Orissa, but it does not seem to have extended beyond the very limited alluvial areas in this district; and, finally, its southerly extension is checked by the sea.

It has been suggested that from the focus in Madras City there has been an extension of the disease in recent years to the surrounding areas, and in a few instances to more distant places in the Madras Presidency. It is true that recently new foci of infection have been discovered in the Presidency, but it has not been possible in most instances to connect these with the original focus in Madras City, and it seems much more probable that they are evidence of the sporadic existence of the disease over a much wider area than was previously recognised. There does not seem to be any evidence that the disease has spread inland for any distance, and here again it appears to be confined to alluvial areas.

The Factors Influencing the Distribution of the Disease

There are certain factors which are common to all the areas in India where kala-azar is endemic; the most important of these can be detailed under five headings, namely: altitude, rainfall, telluric conditions, temperature and humidity.



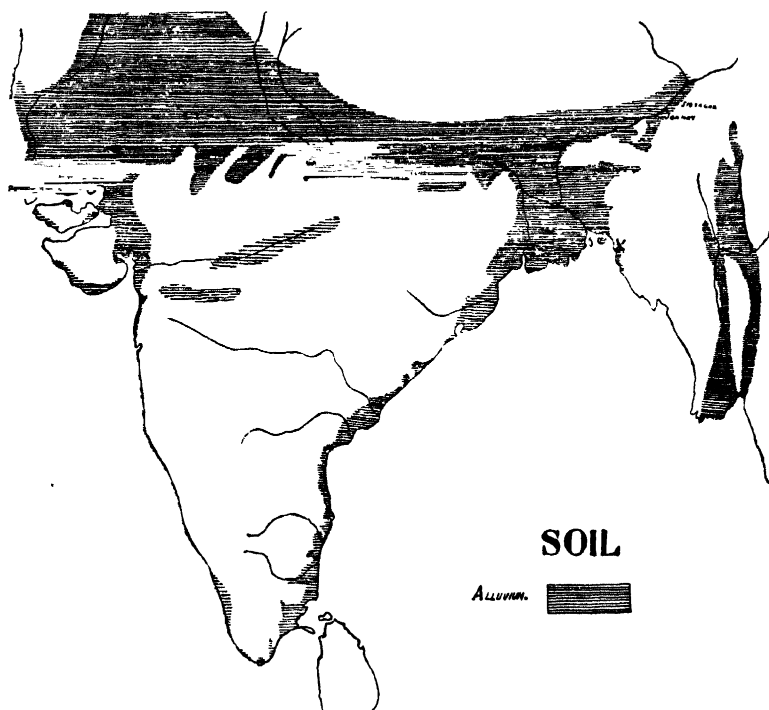
Map III

Altitude.—There is quite a marked correlation between altitude and kala-azar distribution (Map III). It seems to be a fairly well-established fact that kala-azar is not endemic in any area which is 2,000 feet or more above sea level, and, except that there are a small number of cases reported from the Garo Hills and a few cases from the Cachar Hills, the disease seems to be definitely confined to the plains.

Rainfall.—In practically all endemic areas the normal annual rainfall is above 50 inches.

Telluric Conditions.—One of the most striking points about the distribution of kala-azar is that it is confined almost entirely to alluvial soil. Map IV, which is a copy of a map very kindly prepared for me by the Geological Survey of India, shows the alluvial areas.

Temperature.—The temperature conditions common to the highly infected areas are, a monthly mean maximum temperature



Map IV

that is always below 100° F., a monthly mean minimum temperature that is always above 45° F., a mean annual diurnal range of less than 20° F., and a diurnal range of less than 12° F. for at least three months of the year (Chart I). In a few instances in the areas of low endemicity the monthly mean maximum temperature rises to 105° F., and the annual mean diurnal range is as much as 24° F.

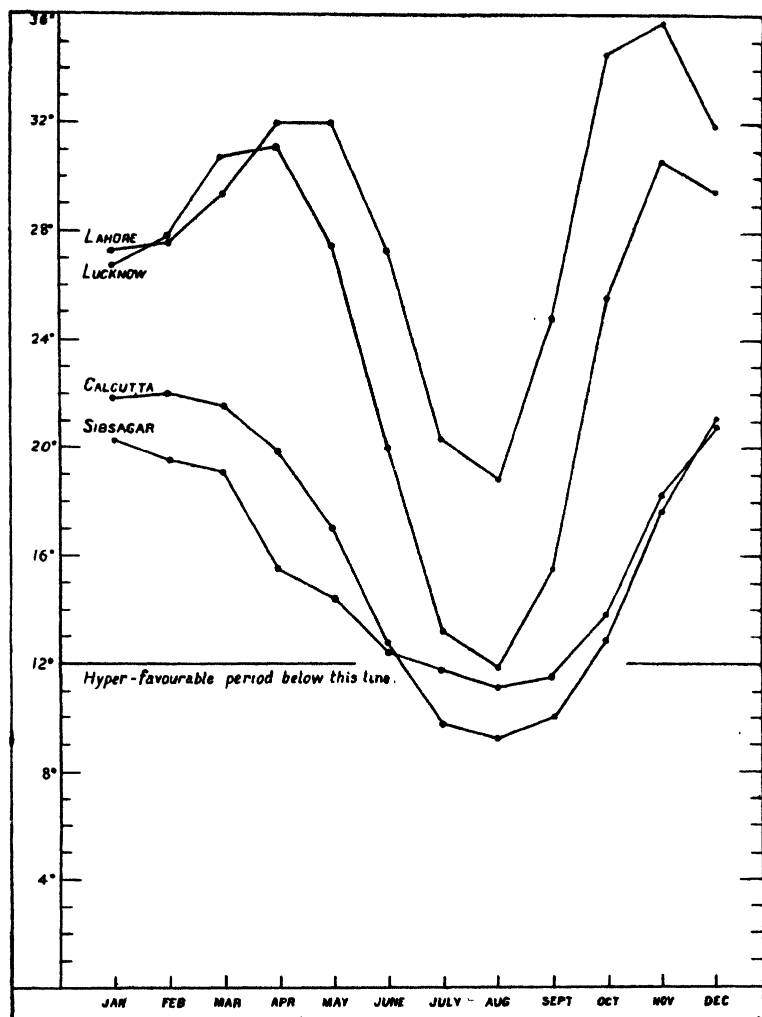
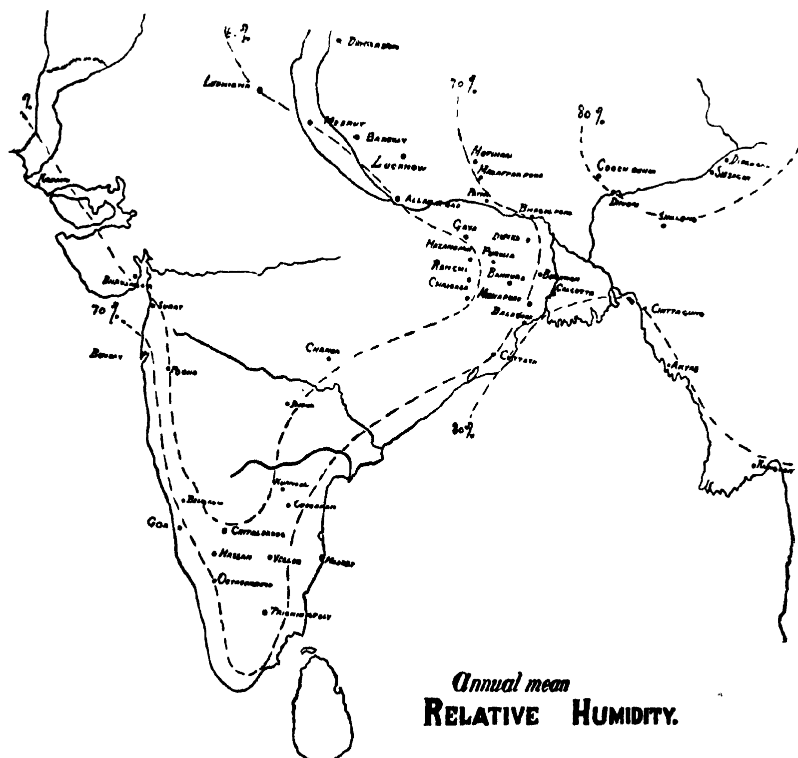


CHART I

Showing the monthly mean diurnal range of temperature throughout the year. Calcutta and Sibsagar, heavily infected places, have a low diurnal range for a number of months during the year. Lucknow, a place from which only an occasional case is reported, has a low diurnal range for only a very short period. And Lahore, which is outside the endemic area, has a comparatively wide diurnal range throughout the year

Humidity.—The important part played by the humidity factor in determining the distribution of the disease has long been recognised; McCombie Young (1924) recently drew attention to this. In Map V lines have been drawn connecting various places which have annual mean relative humidities of 60 per cent., 70 per cent., and 80 per cent., respectively. The common factor of the



MAP V

endemic areas appears to be a degree of humidity which is indicated by an annual mean of daily mean humidities of at least 60 per cent., and for at least three months of the year a monthly mean of eight hours' humidities of at least 80 per cent. The highly infected areas have a degree of humidity which is indicated by a mean annual relative humidity of at least 70 per cent., and a humidity which for

three months of the year is maintained at 80 per cent. throughout the 24 hours.

Other Factors.—Other factors which are common to the endemic areas are a high sub-soil water level throughout the year, a water supply in the rural areas of surface or sub-soil water from tanks or shallow wells, abundant vegetation—with few exceptions—and a rice-growing soil; none of these areas are subjected for any length of time to a direct salt-carrying sea breeze, and neither cotton nor wheat is grown therein.

Although these factors are common to all the kala-azar-infected areas in India, it is fairly obvious that they are not common to the kala-azar areas all over the world. It is possible that the ætiology of the disease is different in other parts of the world, or that amongst these factors which are common in the endemic areas in India there are some which are common to all endemic areas. Exact information about all the endemic areas is not available; most areas are alluvial and of low altitude, but many have not got the same range of temperature; the endemic areas in China, for instance, have a very cold winter, and others are much drier than the endemic areas in India.

Local Distribution

The disease is essentially one of rural districts as against towns, but it does occur to a lesser extent in towns. Napier and Muir (1923) observed that the disease was often rampant in old-established villages sheltered by abundant vegetation, whereas newly-established and more open villages in the immediate neighbourhood were free from the disease. McCombie Young (1924) observed that, although the disease was frequently associated with a primitive type of water supply—usually from tanks or rivers—severe epidemics had occurred in tea gardens with protected water supplies.

The fact that 'site infection' occurs has long been recognised. The removal of a coolie line or village to a new site usually checked the disease in that community for a number of years, whereas the burning down and rebuilding of huts on the old site failed as a preventive measure. Rogers (1899) stated that the removal of a coolie line to a distance of 300 yards from the old site was sufficient to check the spread of the disease amongst the inhabitants.

It is a fairly well-recognised epidemiological phenomenon that

in endemic areas there are 'kala-azar houses', that is to say, houses in which a succession of apparently totally unconnected cases has occurred. The usual history is that one or two cases have occurred in a family, which has then left the district. After the interval of a month or so, another family has come to live in the house, and cases have occurred in the second family. The house has thus got a bad name and has remained vacant for six months or so; after this interval a third family has come to live in the house, and some of its members have likewise fallen victims to the disease.

It has frequently been observed that the incidence of the disease is much greater in the less sanitary coolie lines in a tea garden; and McCombie Young (1924) observed that among the village population in Assam the Kacharis were most heavily infected. These are the most primitive of the peoples of Assam, and usually live amongst very insanitary surroundings.

An investigation into the conditions associated with kala-azar incidence in Calcutta (Napier, 1925) showed that the disease was associated with soil unprotected by pavement or cement; with abundant vegetation and with ground floor residence; also, under certain conditions, with insanitary surroundings, more specially when these these were connected with the presence of chickens or ducks; and with masonry houses rather than *khola* huts.

The areas in Calcutta which are most affected are those that are some distance from the river, the better drained areas close to the river being comparatively free from the disease.

It is a house and family infection; this is possibly dependent on the fact that it is a site infection. McCombie Young reported from Assam that 52 per cent. of cases gave a history of the previous occurrence of the disease in some other member of the household or the family. Rogers (1910) reported that 123 of the 167 relatives of 20 successive cases of kala-azar in the Nowgong hospital had had the disease, and that of these 121 had died.

It has also been pointed out (Rogers, 1910) that young planters have become infected with the disease after coitus with kala-azar-infected women, and that the disease is rare amongst married planters.

The family incidence of the disease is also well marked in Calcutta. On the other hand, the disease does not appear to occur in anything like epidemic form in institutions. Single cases are

frequently reported from schools, but it is usually possible to demonstrate the fact that there was at least an equal chance that the patient was infected in his own home.

Variations in the Incidence of the Disease Year by Year

Unfortunately, our information about the incidence of the disease in any particular district is not sufficiently accurate for us to attach much importance to apparent small variations in the incidence of the disease from year to year. It is doubtful whether the wave of exacerbation of kala-azar that has apparently passed over Bengal during the last few years is a real one; during these few years the medical profession in the province has been educated both in the diagnosis and in the treatment of the disease, and it seems quite probable that this is the real cause of the very large increase in the number of cases that are now reported.

There is little doubt that there was a very marked increase in the incidence of the disease in Assam in 1918-19.

When kala-azar first invaded Assam the arrival of the disease in any particular village usually meant that within a year or so that village, as a village, would cease to exist. It is not suggested that the infection rate and the mortality rate were each 100 per cent., but when the disease had thoroughly established itself the inhabitants faced the inevitable and abandoned the site. It is even reported that not only did they burn down the village and abandon the site, but they burnt all the sick persons and their belongings as well, in order to ensure not carrying the infection to the new site. In other instances, of course, the disease did not get such a firm foothold. When an area had been swept by an epidemic wave, it usually enjoyed a period of comparative immunity from the disease; then, when new generations of non-immunes grew up and some incident lowered the resistance of the community, this area was again the scene of a severe recrudescence of the disease. The Assam recrudescence of 1918-19 was undoubtedly associated with the influenza pandemic which affected Assam very badly in 1918.

In the old-established endemic areas the disease still shows these fluctuations; the incidence in a village will increase year by year until in one year about 10 per cent. of the population is infected, then the incidence will show a gradual decline until, perhaps five years later,



PLATE II

A typical street in the kala-azar endemic area in Calcutta.



PLATE III

A typical compound in the kala-azar endemic area in Calcutta.

there may be only one or two cases in the whole village. This state of affairs may continue for a few years, after which there will again be an increase in the incidence of the disease. Thus in one area the incidence will appear to be declining and in another to be increasing. Bentley (1924) has suggested that kala-azar appears in cycles, and he estimates the period as 10 to 20 years. It is not, however, very clear on what he bases his assumptions; the history of the disease in the past has been much too obscure for any argument to be based on the official reports of the incidence of the disease from year to year. In 1923 the present writer estimated the incidence of the disease in Bengal as being about 1,000,000 cases at a time when the report of the Director of Public Health, Bengal, suggested that it was not less than 50,000. There has been little evidence of any increase in the incidence of the disease during the last few years, and the most recent returns show that in 1925 about 180,000 persons were treated for the disease in the dispensaries and kala-azar treatment centres in the province.

Seasonal Incidence

Rogers observed that the largest number of onsets occurred at the end of the cold weather in Calcutta, and for many years the workers in Assam have noted that a fresh 'crop' of kala-azar onsets appears in the cold weather. The writer analysed the 'date of onset' of a small number of cases in 1921, and found that the greatest number of onsets was recorded in the months of December, January and February.

There are two main obstacles in the way of ascertaining with any degree of exactitude the months or month in which the largest number of kala-azar onsets occurs; these are,

(i) the difficulty which is experienced, even by the intelligent patient, in observing exactly when the first symptoms of the disease make their appearance;

(ii) the inability of the average patient to attach a definite date to any event which occurred more than a few weeks previously.

It is probable that kala-azar, like the acute specific fevers, has a definite incubation period, that is to say, a definite period between the introduction of the causative organism and the appearance of the first symptoms caused by that organism. It is in the nature of

this first appearance of symptoms and in the length of time between this and the subsequent attacks that personal variations are to be observed. In a certain number of cases the onset is

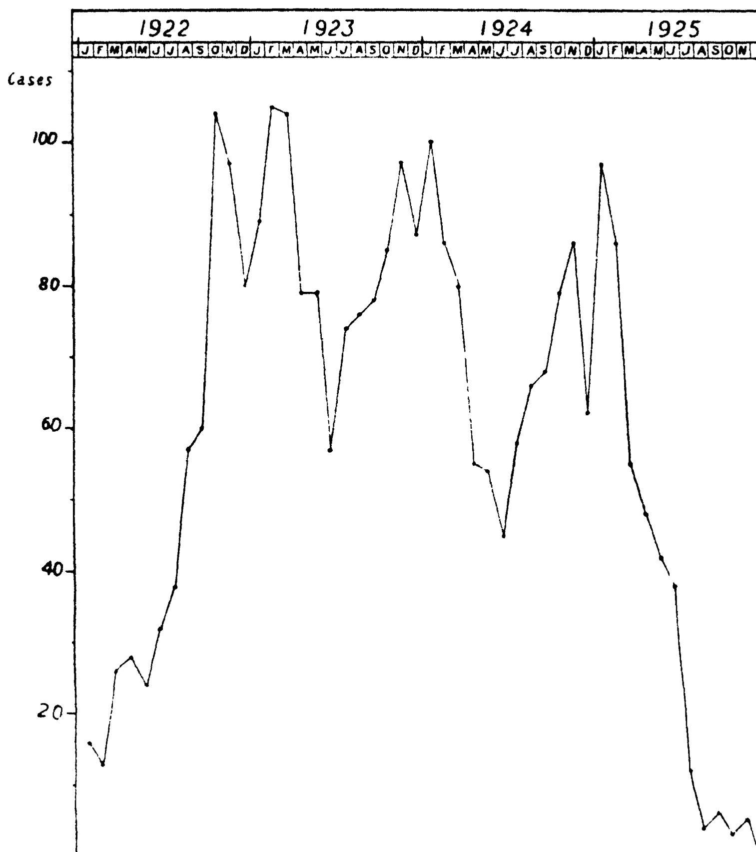


CHART II

Showing the date of onset of the disease. The date of onset of illness of 3,084 kala-azar patients attending the Calcutta School of Tropical Medicine during the years 1923, 1924 and 1925.

marked by an attack of high continued fever, which might be, and in many cases is, looked upon as an attack of typhoid; in other cases this first attack is not so severe, and is diagnosed as malaria,

influenza or dengue; and, finally, probably the greatest number of patients have an attack of fever so slight that, either the incident does not impress itself on their memory for any length of time, or it is actually not observed by them. After this onset the patient usually enjoys a period of comparatively good health. The length of this period seems to vary very considerably in different persons: it is sometimes only a week or so in duration; we have known instances in which it was five months, and it is probable that, occasionally, it is a much longer period than this. It is this second attack that impresses itself on the patient's memory, and it is by no means uncommon for a patient to assure one that he has been perfectly well until a fortnight ago, when the condition of his blood suggests that he has been suffering from the disease for at least five months. Thus, supposing for the moment that all infections took place during three months of the year, even if one could rely on the statements of the patients, the histories of onset which one would receive would be of dates extending over a period of seven or eight months; and, further, if one took into account the gross inaccuracy of the histories which are given by patients—especially with reference to events which occurred a year or so previously—one would expect the recorded dates of onset to be spread over the whole year, with probably the largest number at the actual months of onset and the few months following these.

Recently, the author analysed the case cards of the kala-azar out-patients' department of the School of Tropical Medicine, with reference to the month of onset as given by the patients. The cards of all patients who were definitely diagnosed as kala-azar—by means of either spleen puncture or the aldehyde reaction—and who attended first during the years 1923, 1924 or 1925 were analysed. The total attendance of kala-azar patients during these years was 1,117, 1,118 and 1,108 respectively; it is thus obvious that the attendance was not undergoing a steady increase, as was the case during the first two years that the department was open. As it seemed possible that this increase might influence the onset curve, the patients who attended during these two years were not included in the analysis. In certain instances the patient could give only the year of onset and not the month, and in a few others no note was made on the card, so that the total number of cards analysed was only 3,084. Chart II shows the month and year of onset of these 3,084 patients.

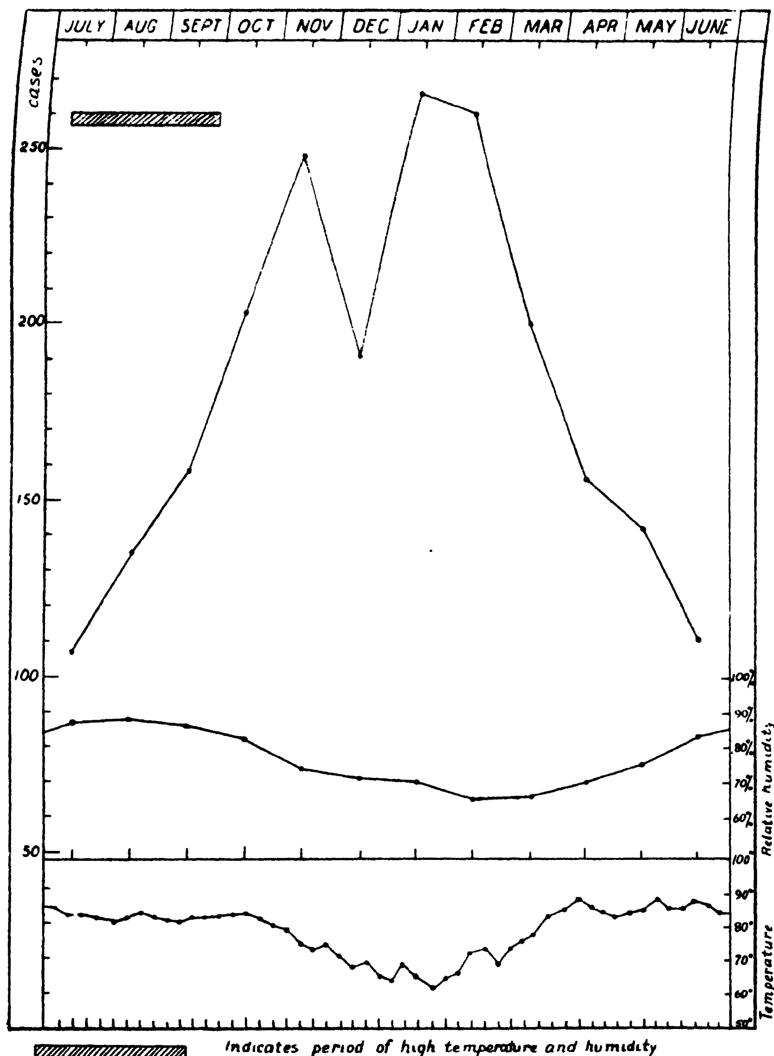


CHART III

Graph of the month of onset in relation to the humidity and temperature. The onset curve is based on the histories in 2,130 cases in which the disease was of less than one year's duration; the temperature curve is based on the weekly mean temperatures; and the humidity curve on the monthly mean relative humidities.

In the case of patients giving histories of six months' illness the error probably amounts on an average to one or, at the most, two months in either direction, but in the case of those giving a history of one year's illness the chances of error are very large indeed. Now, were the histories accurate, the onset curves for the patients who had waited a year or more before coming for treatment would be a repetition of the onset curve of those who had come for treatment within the first year of illness; we ought, therefore, to be able to ignore all cases with a history of a year or more's illness without distorting the onset curve.

Chart III shows the month of onset of 2,130 cases in which there was a history of less than one year's illness. The exclusion of cases with a history of illness of one year or more does not alter the general character of the curve, but brings out the contrast rather more sharply between the months May to August, which show the smallest number of onsets, and the months November to February, in which the greatest number of onsets is reported.

A separation of the case cards according to whether the patients came from Calcutta or the various districts of Bengal did not show that the seasonal incidence varied in different places.

The outstanding feature of all these curves is the sharp rise which commences in September, that is to say, from two to three months after the beginning of the monsoon. The two factors which are likely to cause seasonal variations in the incidence of a disease are seasonal variations in the habits of the community, and seasonal variations in the climatic conditions. It is difficult to see how the former factor could account for this rise in the onset curve, although it is conceivable that, in certain districts which are subject to annual flooding, the insanitary crowding of the population which occurs on small areas of dry land might facilitate the transmission of the disease at this season; however, the post-monsoon onset is just as frequently observed in areas which are not subject to this flooding. It seems probable, therefore, that the latter is the factor responsible.

It is obvious that the only important climatic event in the few months before the sudden rise in the onset curve is the arrival of the monsoon, with its effect on the temperature and humidity. Monsoon conditions are usually well established by the middle of July in the principal endemic areas; the humidity is high, seldom falling below 80 per cent. in the 24 hours, and the temperature, which

has a daily mean a little above 80°F., shows only a small diurnal range. These conditions are maintained for at least three months. Temperature and humidity curves have been added to Chart III; these demonstrate graphically the conditions preceding the rise in the onset curve.

It is probably during these three months that the majority of the kala-azar infections occur.

It is quite easy to reconcile this assumption with the onset curves that have been shown above, if one assumes that the incubation period is about two months and takes into consideration the indefinite nature of the onset in most cases and the inaccuracies of the histories given by the patients.

AGE INCIDENCE

Figures have been collected from four different sources in India:

1. A village treatment centre near Calcutta (Basu, 1924).
2. Assam (McCombie Young, 1924).
3. The out-patient department of the Calcutta School of Tropical Medicine (Napier, 1926*a*).
4. Southern India: Madras Presidency (Turkud *et alia*, 1926).

In the case of the Bengal village treatment centre the diagnosis was clinical only; the disease in children is clinically much more typical than it is in adults, so the error here would probably be that not sufficient adults were included. On the other hand, there would not be any errors in sampling from other causes. In the Assam cases again, the diagnosis would be largely clinical, but in the instance of the out-patient department of the School of Tropical Medicine, although the patients—many of whom come from some distance—will have undergone a certain amount of selection, the diagnosis was more accurate. The diagnosis depended in about half the cases in the last group on spleen or liver puncture, but as 103 of 113 punctures that were carried out were 'positive', it seems possible that the selection of cases was not quite wide enough; this would account for the small percentage of adults in this series, adults being clinically less typical than children.

Table I shows these four sets of figures.



PLATE IV

A group of patients awaiting treatment at a village treatment centre.

TABLE I

	Bengal village figures	Assam dispensary figures	Calcutta out-patient figures	Southern India figures
	%	%	%	%
Under 1 year	..	0·06	0·26	
Under 5 years	8	9·3	4·5	5·1 (under 3 years)
5 years or more, but under 10 yrs.	29	21·4	16·4	37 (3 to 10 years)
10 " " " 15 "	23	20·5	23·8	44
15 " " " 20 "	17	17·3	17·3	
20 " " " 30 "	14	18·0	22·6	10·5
30 " " " 40 "	7	9·7	10·0	
40 years or more	2	3·8	5·2	3·4
Number of cases on which percentage is based	1,599	16,524	3,123	216

The distribution of cases amongst the various ages seems to be much the same in all four sets of figures, and it is easy to believe that the slight differences that do exist are due to the selection which the cases underwent on the one hand, and error in diagnosis on the other. The greatest number of cases is between the ages of 5 and 15 years, and infants seldom appear to be infected.

The age incidence graph for 2,106 Calcutta cases has been drawn on Chart IV. The curve has been made very irregular by patients giving their ages in round figures, but it is fairly obvious what the real curve should be. It rises sharply to the 12-year mark, and then falls fairly rapidly to the 40-year mark.

Young (1923) collected 762 cases from various sources in China; he found the age distribution as follows:

TABLE II

Years	Percentage	Years	Percentage
1-10	.. 31·5	31-40	.. 7·9
11-20	.. 40·1	41-50	.. 1·8
21-30	.. 18·4	51-60	.. 0·3

Allowing for the slight difference in the method of grouping, it is obvious that the age distribution in China is almost exactly the same as in India.

Only a small number of figures are available from the Sudan. Bousfield (1911) states that of 22 patients the average age was 18, that the oldest was about 40, and the youngest 6 years old. Thomson (1911) states that out of 37 patients from the Blue Nile district 24 were between the ages of 7 and 18, but that out of 5 patients from Kassala 4 were adults. Here, again, the age distribution cannot be said to differ markedly from the age distribution in India.

In the Mediterranean type of the disease, however, the age incidence is entirely different. From Tunis, Nicoll (1925) reports that 37, or 55 per cent., out of a total of 67 patients, were between the ages of 1 and 3 years, and that the oldest was 9½ years. In Pittaluga's (1925) Spanish series, out of 332 cases, 68 per cent. were 3 years or under, and only 1·5 per cent. were over the age of 10. This is in very marked contrast to the state of affairs in India, where, on an average, 70 per cent. of the patients are aged 10 years or over. Pittaluga's series appears to be fairly typical of the Mediterranean type of the disease; it is given below in detail.

TABLE III.—AGE INCIDENCE IN SPAIN

Age	Number	Percentage	Age	Number	Percentage
Less than 6 months	11	3·3	5 to 6 years	16	4·8
6 months to 1 year	36	10·8	6 to 10 years	3	0·9
1 year to 2 years	120	36·1	10 to 12 years	1	0·3
2 to 3 years	58	17·4	12 to 15 years	2	0·6
3 to 4 years	48	14·1	23 years	1	0·3
4 to 5 years	35	10·5	46 years	1	0·3
			Total	332	

SEX DISTRIBUTION

Figures for the Assam treatment centres show that 70 per cent. of those attending are males. One would expect a certain amount of hesitancy on the part of the inhabitants in bringing out their adult women for treatment, but one would not expect it in the case of the female children. McCombie Young (1924) is of the opinion that

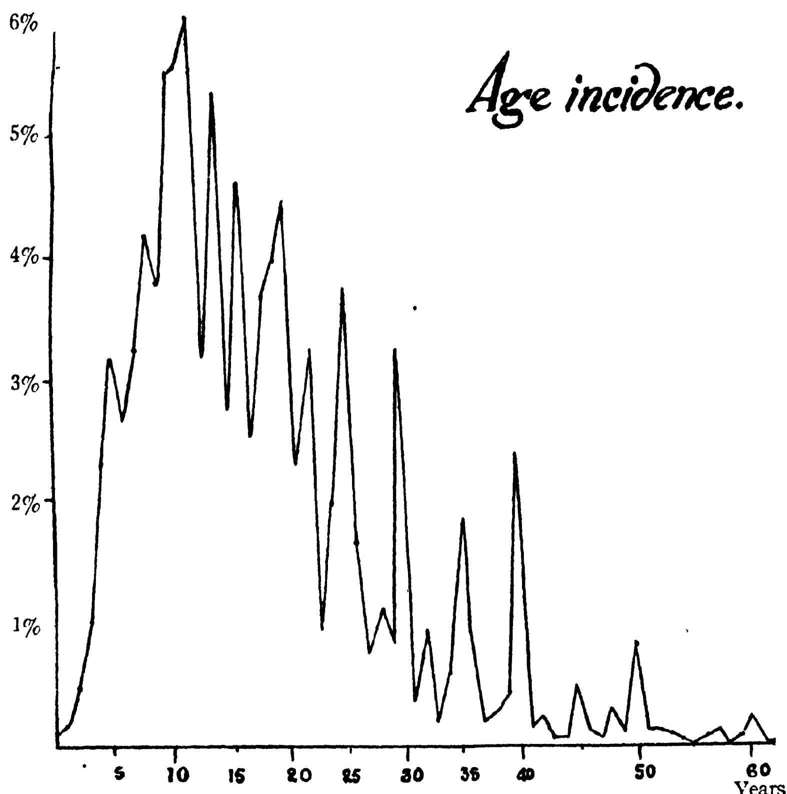


CHART IV

Chart showing the ages given by kala-azar patients attending the Calcutta School of Tropical Medicine.

there is a distinct predominance of males, but that this predominance is not as great as the figures indicate.

Analysis of the out-patient figures shows that 76 per cent. of the patients attending the School of Tropical Medicine are males. The hesitancy to bring adult females for treatment would be much more marked when they have to be brought some distance, and the writer is not satisfied that the Calcutta figures indicate that more males than females become infected.

Race, Religion and Caste Distribution

The disease is rare amongst better class Europeans; a few cases have come to our notice in Calcutta during the last few years, and the European planter, living in a comparatively well-kept bungalow on a tea garden in Assam, is occasionally attacked. However, the disease is not at all uncommon amongst pure Europeans living under conditions similar to those under which the poorer Anglo-Indian usually lives.

The disease shows a relative predominance amongst the Anglo-Indian and Indian Christian communities, as far as Calcutta is concerned.

We see a comparatively large number of Nepalese patients amongst our Calcutta cases, but it would be difficult to be certain that the number was disproportionate to the number of Nepalese living in Calcutta. It is, however, noticeable that they get the disease much more acutely than the indigenous population.

In Calcutta it is observed that Muhammadans are more frequently attacked than Hindus, if allowance is made for the relative numbers of each class in the town, but the cases that come to us from outside the town are almost all Hindus. Hindu villages appear to be as frequently attacked as Muhammadan villages, but in mixed villages if there is any difference it is usually the Muhammadans who suffer most.

Persons of all castes suffer from the disease, but there appears to be a slight relative predominance of the sweeper castes amongst the patients.

To summarise, one may say that the racial, religious and caste distribution of the disease is fairly even; under certain circumstances one class predominates and under different circumstances another, so that any argument based on the observation of the predominance of any class amongst the patients of one locality will be unsafe unless the special conditions in that locality are taken into consideration.

No occupational prevalence is observed.

CHAPTER II

ÆTIOLOGY

The causative organism—History of discovery—Morphology—Infectivity of the flagellate—The transmission problem—Theoretical considerations—The theory of contaminative transmission—The theory of insect transmission—The bed-bug—Fleas—Lice—Reduviid bugs—Ticks—Mites—Stomoxys and tabanids—Mosquitoes—Sandflies—Culicoides—Epidemiological evidence—Experimental evidence—Experimental animals—Summary of conclusions regarding the kala-azar transmission problem—The importance of the secondary factor in kala-azar transmission—Phlebotomus argentipes—Geographical distribution—Habitat—Prevalence—Feeding—Flight—History of the first incrimination of the sandfly—The probable life cycle of *L. donovani* and the probable mechanism of the infection of man.

THE CAUSATIVE ORGANISM

POSITION IN THE PROTOZOOLOGICAL CLASSIFICATION

THE causative organism of kala-azar, *Leishmania donovani*, is a protozoon. Its exact position in the protozoological classification has been a matter of discussion for some time. Wenyon's (1926) classification is as follows: sub-phylum—Plasmodroma, class—Mastigophora, sub-class—Zoomastigina, order—Protomonadida, family—Trypanosomidæ, genus—Leishmania, and species—Donovani. The other members of this genus are *Leishmania tropica*, *Leishmania braziliensis* and *Leishmania canis*. Recent work by Noguchi (1925) suggests that the causative organism of infantile kala-azar is serologically identical with *L. donovani*.

Some protozoologists claim that the causative organism of kala-azar is a true herpetomonad, and that the name *Herpetomonas donovani* should be applied to it. Their claim is based on the fact that other herpetomonads are capable of completing their life cycle in a vertebrate host; the case of *Herpetomonas davidi*, which infects euphorbiaceæ, and the claims of Fanthom and Porter (1915) and others to have infected small mammals with *H. ctenocephali* are quoted, although Hoare (1921) and others (Shortt, 1923*a*) have failed to confirm these last findings. Against this it is urged that the life cycle of *L. donovani* is not similar to that of other known

herpetomonads, although morphologically it is similar in its flagellate stage to other members of the genus Herpetomonas. The position as it stands at present is that, although those who claim the unity of the two species may be right, as their case is not sufficiently strong to warrant interference with the established nomenclature, the genus Leishmania remains valid. It is probable that this parasite was originally an insect flagellate, and that the infection of man was more or less accidental and not an essential part of its life cycle. Whether the insect in which it was originally the natural flagellate is the spreader of the disease, or whether the disease is spread by another insect whose infection was consequent on the accidental infection of man, it is not possible to guess.

HISTORY OF DISCOVERY

Although for some time kala-azar has been recognised as a communicable disease, it was not until April, 1903, that the causative organism was described almost simultaneously by Leishman (1903) and Donovan (1903), the former having noted it in the spleen of a soldier who had died of a long-continued fever, which he had contracted in Bengal, and the latter in the post-mortem spleens and the spleen pulp obtained from living patients in Madras. Very shortly after the discovery of the parasite in Indian kala-azar cases Pianese (1905) found similar bodies in the spleens in cases of infantile kala-azar in Italy, and Wright (1904) in the scrapings of 'Delhi boil'.

Rogers (1904) was the first to culture the organism and thus discover its flagellate phase; he found, by placing the material obtained by spleen puncture from a case of kala-azar into normal saline to which 5 per cent. sodium citrate had been added and incubating at 22°C., that the bodies remained alive, enlarged and eventually developed into typical herpetomonads.

MORPHOLOGY

Two phases of the parasite are known, the 'round', or aflagellate, and the flagellate phase.

The Aflagellate Form, or Leishman-Donovan Body.—This phase occurs only in the body of the mammalian host. The parasite is usually observed lying within a cell, singly or in large numbers, but extra-cellular forms are also observed; they are

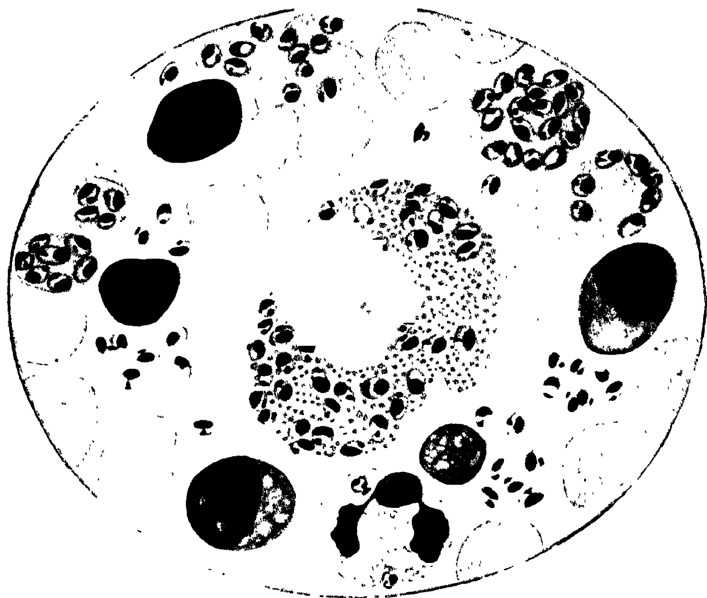


Plate XII.
Leishmania in spleen puncture smear, (after Knowles).



present in the polymorphonuclear and large hyaline cells of the blood, and in endothelial cells in the organs and other parts of the body, but they seldom appear to invade the parenchymatous cells of the organs.

In this form it is an oval or round body with an average diameter of about 2μ, in the oval forms the breadth being about three-fourths or four-fifths of the length. It consists of cytoplasm containing a nucleus which is more or less round and a little less than 1μ in diameter, a parabasal from which springs a rhizoplast, and a vacuole.

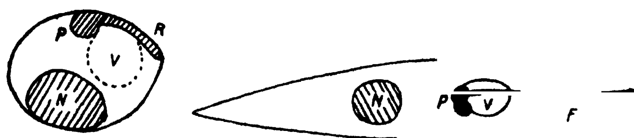


FIGURE I

The structure of the Leishman-Donovan body and the flagellate form of *L. donovani.* (After Christophers, Shortt and Barraud, 1926.)

N. nucleus.
P. parabasal.
R. rhizoplast.
V. vacuole.
F. flagellum.

The Flagellate Form.—The flagellate form is observed in certain insects, most notably in sandflies, and in culture medium. It shows a number of morphological variations, but, generally speaking, it is a fusiform organism with a flagellum. The length of the body is from 5μ to 15μ, the breadth from 2μ to 0·5μ, and the length of the flagellum from 10μ to 15μ. The body of the flagellate consists of cytoplasm, a centrally situated nucleus, a parabasal situated about midway beween the nucleus and the anterior end of the body of the parasite, a rhizoplast and flagellum springing from the parabasal, and a flagellar vacuole lying between the parabasal and the anterior end of the body of the parasite.

MULTIPLICATION

Multiplication of the aflagellate forms occurs within the cells of the body of the mammalian host. When, however, the parasite is

transferred to suitable culture medium, or is taken into the gut of the suitable insect host, the 'round' form develops into a flagellate form.

The process of division of the flagellate occurs as follows: The parabasal becomes bi-lobed and eventually divides; the rhizoplast then divides and a new flagellum is formed; the nucleus divides; in the meanwhile the whole body of the flagellate becomes shorter and broader, and now divides into two pear-shaped bodies.

When the mature flagellate has formed from the Leishman-Donovan body, it comes to rest in some suitable spot; here active division occurs until a large group has formed; from this group individuals detach themselves, swim away, eventually come to rest, and the process is repeated. Whether this process is continuous or whether an end-stage is eventually reached is not clear; it seems possible that the long slender forms which eventually develop represent the final stage, and that these forms do not undergo further division in culture medium or in the gut of the hypothetical insect transmitter, but are the infective forms which are capable of continuing the cycle in man. The Kala-azar Commission (1926) have made a very careful study of the life cycle of the parasite in culture medium, and have described a number of different types of flagellates which represent different stages in development; they have observed that a slender form and a cyst-like form, which latter they suggest may be the infective form, do not appear in cultures until about the seventh day.

BIOLOGY

The flagellate grows most readily in the presence of hæmoglobin. The best artificial medium for its growth is one consisting of water, agar, sodium chloride and rabbit's blood. This medium has a hydrogen-ion concentration of about pH 7; development will, however, occur in the presence of a much higher or of a much lower concentration of hydrogen-ions (Napier, 1924). Small changes in the osmotic pressure of the medium are not fatal to the parasite, but it will not live for any length of time in water (Noguchi, 1924) or in milk (Kala-azar Commission, 1925).

The presence of bacteria in culture medium is usually fatal to the parasite. Ordinary human serum kills the parasites, but they will survive for some time in the presence of de-complemented human serum.

Development occurs at temperatures between 18°C. and 30°C., and the optimum temperature would appear to be about 26°C., and not 22°C. as has previously been supposed.

SEROLOGY

Noguchi (1924), by injecting rabbits with cultures of flagellates, was able to produce a serum which would cause complete agglutination of homologous strains in dilutions of 1 in 10 and partial agglutination in dilutions of 1 in 100. He found that *L. donovani* and *L. infantum* were serologically identical, but that these two species were serologically distinct from *L. tropica* and *L. braziliensis*, these latter two being distinct from one another.

THE INFECTIVITY OF THE FLAGELLATE

Row (1922) put forward the suggestion that the post-flagellate, or O-body, was the infective stage; these bodies, he claimed, were only found in drying cultures. The Kala-azar Commission (1925) were able to produce infection in tame mice with a considerable degree of regularity by the injection of flagellate cultures more than 7 days' old, but they failed to produce infection by the injection of younger cultures; they thought that the aflagellate, cyst-like forms present in the older cultures only were probably the infecting forms.

The present writer, recently, by giving single injections of young (5-day) primary cultures, has produced leishmaniasis in young white mice. The age of the culture does not appear to be an important matter; the failure of the above-mentioned workers to infect mice with young cultures was probably due to the scantiness of the parasites in these cultures.

THE TRANSMISSION PROBLEM

Although the causative organism of kala-azar has been known for the last 23 years, the means by which man becomes infected by this organism is not yet known: the problem of how the disease is transmitted may be said to be the most important of the unsolved problems of tropical medicine. The problem has been tackled by a number of men whose names are well-known in the annals of tropical medicine, but, until quite recently, little light has been thrown thereon. A number of suggestions have been made

and a number of insects have fallen under suspicion as possible transmitters, foremost amongst which was the bed-bug, but the evidence in each case has been so slender that recently there has been a tendency amongst those engaged in the problem to break new ground, to look for insects which have hitherto not come under investigation, or even to abandon the insect theory of transmission altogether. Recently, the investigations at the Calcutta School of Tropical Medicine and, later, those of the Kala-azar Commission working in Assam have incriminated the sandfly, *Phlebotomus argentipes*; the evidence against this sandfly is so strong that at the time of writing, although actual proof is wanting, there does not seem to be any possibility that this proof will not shortly be forthcoming.

However, in view of the fact that the question of the means of transmission is still an open one, it will be as well to review the problem and to state the various possibilities in connection with it.

ANALOGY

In considering a problem of this nature we are, to a very great extent, influenced by analogy; even the suggestion that the disease may be transmitted by an arthropod is dependent on analogy.

For the complete treatment of the problem from this point of view it would be necessary to review the life histories of all protozoa; this is, of course, out of the question, but it will perhaps not be out of place if we go into a few points of analogy.

No instance of infection of man by a herpetomonad—if leishmania be excluded—is known, but the infection of a dormouse with *Herpetomonas myoxi.*, of lizards with *H. tarentolæ* and *H. hemidactyli* and of euphorbiaceæ with *H. davidi* have been reported; it is assumed that the blood infections in the lizard were dependent upon prior intestinal infections, but the plant herpetomonas is reported by Franca (1920) to be transmitted by the plant-bug, *Stenocephalus agilis*.

There are a number of natural herpetomonad infections of arthropods; many of these have been responsible for misleading some of the earlier workers on the kala-azar transmission problem. Roughly speaking, the life cycles of these flagellates can be divided into two groups: those that are transmitted from one imago to another by the latter feeding on material infected by the fæces of

the former, as is the case with *H. muscarum*; and those that are transmitted by the larvæ ingesting some substance infected from the fæces of the adult, as in the cases of *H. culicis* and *H. ctenocephali.* In both instances the aflagellate stage only is passed outside the body of the arthropod.

The frequency with which members of this genus have been observed in arthropods has naturally led to the suggestion that members of the closely allied genus Leishmania pass their extra-corporeal stage in the gut of some arthropod.

Furthermore, many specific suggestions have been put forward on the grounds of analogy. The fact that *Trypanosoma cruzi*, the causative organism of a disease which ætiologically has some points in common with kala-azar, and an organism which bears a certain resemblance to *L. donovani*, is transmitted by *Conorhinus megistus*, a species of Triatoma, has very naturally led to the suggestion that *Conorhinus* (*Triatoma*) *rubrofasciatus*, a species which is known to exist in most endemic areas in India, is responsible for the transmission of kala-azar.

Recent work on oriental sore has incriminated a sandfly, *Phlebotomus papatasii*, as the intermediate host and transmitter of *Leishmania tropica*, the causative organism of this condition. It is strongly suggestive that, if the sandfly which is the one commonly observed in the drier alluvial areas of the Indo-Gangetic plain transmits the form of leishmaniasis endemic in these areas, the sandfly common to the more humid areas of this plain, namely, *P. argentipes*, should transmit the form of leishmaniasis endemic in the latter areas.

There is, however, a danger of being unduly influenced by considerations of analogy, and we must pass on to a systematic review of the problem.

THEORETICAL CONSIDERATION OF THE POSSIBLE MEANS OF TRANSMISSION OF THE DISEASE

There are three main possibilities with regard to this problem, namely:

(*a*) that the disease may be transmitted from man to man, either directly, as in the case of most bacterial diseases, or indirectly by the aid of an insect, as in the case of malaria;

(*b*) that it may be transmitted from another vertebrate which

acts as a reservoir or alternative host, either directly, as in the case of many helminthic infections, or indirectly, as in the case of plague;

(*c*) that it may be transmitted from an insect in which the flagellate is a natural parasite, and in which it is capable of completing its life cycle independently of man or other vertebrate.

If the disease is transmitted by either of the last two means, the suggestion is that man is not essential for the completion of the life cycle of the parasite. It is a widely accepted epidemiological hypothesis that infected man is an essential factor in the transmission of the disease, but investigations to prove this hypothesis can only be satisfactorily carried out at the spreading edge of the infection. Most investigators seem to have been satisfied that the introduction of the disease into a locality is always caused by the importation of an infected person, although in many instances reported in the literature the evidence has been of a hearsay nature from villagers. The reverse is certainly not true; the introduction of infected cases even in large numbers has not by any means always been followed by the incidence of the disease in that locality, even in places which subsequent history has shown to be potential endemic areas. The best argument in favour of this hypothesis is the statement that intensive treatment will check the disease in a community. This again is a statement which has not yet had a thorough and unprejudiced examination. The appearance of the disease in epidemic form in a village or a tea garden coolie force is, in the natural course of events, followed by a number of years of comparative freedom from the disease, and, as stated above, in established endemic areas the disease is liable to appear in cycles. The apparent infectious nature of the disease and the fact that house infection, to which reference will be made later, is common, is not an argument in favour of this hypothesis, as house infection could be produced by the presence of an animal reservoir or of a number of naturally infected insects.

Although the evidence is on the whole in favour of an infected man being an essential factor in the transmission of the disease, we must be content with a verdict of 'not proven'; as, however, it is most probable that the disease is transmitted from man to man, the possible means by which this might occur are worthy of detailed consideration.

TRANSMISSION OF THE DISEASE FROM MAN TO MAN

This must be considered under three headings, namely: (*a*) the route by which the parasite leaves the body of the host; (*b*) the route by which it gets entry into the new host; and (*c*) the means by which it is carried from the original host to the new host.

THE POSSIBLE ROUTES BY WHICH THE INFECTING ORGANISM COULD LEAVE THE BODY OF THE HOST

(*a*) By means of excretions or secretions.

(*b*) From the peripheral blood by the agency of a blood-sucking insect.

Most of the possibilities included under the first heading have been investigated; the only two routes that are worthy of consideration are the intestinal and the urinary tracts.

Infection from the Intestinal Tract.—As dysentery and diarrhœa are common complications of the disease, the escape of the parasite by this route is suggested. Critien (1910), Mackie (1914), and Knowles (1920) described protozoal-like bodies in the fæces. There seems to be little doubt that these bodies were yeasts; Knowles, Napier, and Gupta (1923) noted that of 265 stools of 210 kala-azar patients, in 57 per cent. yeasts were a very prominent feature; whereas in 456 stools of patients taken from the same area, but who were not suffering from kala-azar, this degree of prominence was only noted in 10 per cent. These protozoal-like bodies have been encountered in the stools of kala-azar patients and of other patients not suffering from the disease, but more frequently in the former; this is probably on account of the fact that yeasts of all varieties are present in greater variety in the stools of kala-azar patients.

Marian-Perry (1922) recorded the finding of *L. donovani* in large numbers in the jejunum in two cases of kala-azar; there had been considerable proliferation of endothelial cells in the villi, and these cells were filled with parasites. The epithelium of the villi was absent, so that it seemed possible that this demonstrated a means by which the parasite could be eliminated. Meleney (1925), however, points out that the loss of the epithelium was probably a post-mortem event, as the epithelium is intact at the base of the villi; he has shown that the same condition is found in the kala-

azar-infected hamster, but that if precautions are taken in preparing the specimen the epithelium is always found intact. He has never observed the parasites in the epithelium. It would thus appear that, unless there is ulceration, it is not possible for the parasites to escape into the lumen of the gut.

If, however, the infecting organism escapes from the body by this route, there must necessarily be a more resistant stage in the life cycle of the parasite than any with which we are familiar. In culture medium we know that the presence of bacteria will in every instance prevent the development of the parasite, and the introduction of bacteria into a culture-tube will eventually lead to the death of the leishmania, although in some instances they appear to resist the effects of bacterial contamination for 48 hours.

Infection from the Urinary Tract.—Shortt (1923*d*), by placing the centrifugalized deposit of a catheter specimen of the urine of a kala-azar patient on N.N.N. medium, demonstrated that viable forms of the parasite were sometimes passed in the urine. Napier and Gupta (1923) failed to obtain a growth from the sediment of kala-azar patients' urine which had not been centrifugalized, but were able to do so when one drop of the patient's peripheral blood was added to a test-tube full of urine; they concluded that the presence of the parasite was an accident dependent on the presence of blood or other leishmania-infected cellular deposit in the urine.

Infection from the Peripheral Blood.—The fact that the parasite is always present in the peripheral blood of a patient suffering from kala-azar gives support to the hypothesis that during transmission the parasite leaves the host by this route.

THE POSSIBLE ROUTES BY WHICH THE NEW HOST COULD BECOME INFECTED

The only two routes which need be considered are infection by the mouth, and infection through the skin, invasion by the latter route occurring through an abrasion or by inoculation by a blood-sucking insect.

Invasion by the Mouth.—Before considering the possibility of infection occurring by ingestion, we must presuppose the resistant stage in the life cycle of the parasite. The Kala-azar Commission (1925*a*) have shown that the flagellate form will resist

a certain degree of variation in the osmotic pressure of its environment, but that there is little chance of its survival in water or even milk for any length of time, and it is therefore probable that the flagellates would not withstand the various osmotic pressures to which they are likely to be subjected in the stomach and the intestinal canal; Napier and Murugesan (1924), on the other hand, showed that the parasite would not only live but would develop in environments with hydrogen-ion concentrations of pH 4·5 to pH 8·8, and concluded that the hydrogen-ion concentration of the contents of the stomach of the majority of rice-eating Indians, and of young children of most races, would not prevent the development of the parasite therein.

Experimental Evidence.—There are in the literature at least four instances in which animals have been infected with kala-azar apparently through the ingestion of infected material. Archibald (1914) reported the instance of a monkey becoming infected after being fed on leishmania-infected material; Cornwall and LaFrenais (1916) reported the instance of the infection of a white mouse by this method; Knowles, Napier and Das Gupta (1923) reported the instance of infection occurring in a monkey (*Macacus rhesus*) which had been fed with an emulsion of the spleen of a patient who had died of kala-azar; and, finally, Greig and Christophers (1925) reported the instance of a monkey, also *Macacus rhesus*, becoming infected with kala-azar four months after having spleen puncture material and a culture of leishmania inoculated directly into the lumen of the small intestine.

Recently, the Kala-azar Commission (1926 *a* and *b*) failed to infect any of a large series of mice by feeding them on cultures of leishmania, whereas they produced infection in almost 100 per cent. when the cultures were injected into the peritoneal cavity.

These 'positive' experiments are four out of a very large number of 'negative' ones, and in no instance were they carried out under conditions which could conceivably be reproduced in nature; nevertheless, they show that invasion can occur through this route.

Infection through the Skin.—If infection occurs by this route the most probable means is through the bite of a blood-sucking insect. Under these circumstances the inoculation would be intradermal.

Experimental Evidence.—The examples of infection having been

produced by this means are very few (Shortt and Swaminath, 1925*a*), but it must be remembered that if Besredka's theory of the specificity of the susceptibility of groups of cells to special organisms can be applied in the case of the protozoa as well as the bacteria, and if an intradermal inoculation is an important point in the production of infection, it is quite easy to understand why difficulty has been experienced in producing infection in such small animals as mice; in these thin-skinned animals it is almost impossible to inject any quantity of material intradermally. Infection can be produced in monkeys and mice with a considerable degree of regularity by the intraperitoneal injection of either a large quantity of heavily infected spleen-juice or a smaller quantity of flagellate culture.

On one occasion the writer apparently produced kala-azar in a man by the cutaneous inoculation of spleen puncture material from a kala-azar patient; but, on the other hand, on two occasions he has run the point of a needle, immediately after withdrawal from an infected spleen, deeply into his own finger, and has, up to the present, that is, at least two years after the second occasion, not developed kala-azar.

On the whole, however, the experimental evidence cannot be said to be any more strongly in favour of this as a means of introduction of the parasite into the new host than it was of the alternative means, i.e. by the mouth.

THE MEANS BY WHICH THE PARASITE MAY BE CARRIED FROM THE ORIGINAL HOST TO THE NEW HOST

The simplest way will be to consider the possibilities under two main headings, namely: (*a*) transmission from man to man independently of a blood-sucking arthropod in which the parasite lives an essential part of its life cycle or undergoes development—this we might call the theory of contaminative transmission; and (*b*) transmission by means of a blood-sucking arthropod in which the parasite passes an essential part of its life cycle or undergoes development—this we might call the theory of insect transmission.

The Theory of Contaminative Transmission.—If transmission occurs this way, then one must assume that the infecting organism comes from the excreta of the patient and is

ingested by the new host, the transfer of the material occurring either directly, by means of exhalations, touch or contaminated clothing; or indirectly, by the contamination of some medium such as water, milk, or food by any means, including the contamination by an insect—such as the housefly—which merely acts as a mechanical transmitter.

On theoretical grounds this possibility is negatived, as the transmission of diseases which are transmitted directly from man to man is to a large extent independent of environmental conditions. It is therefore unlikely that diseases such as kala-azar, which have a strictly limited geographical distribution, are transmitted by this means. (Actual experience appears to have disproved this theory; yaws, for example, which is probably only transmitted by direct contact, appears to have a very limited geographical distribution. It does, however, seem probable that temperature and humidity are the only factors which can influence diseases which are thus transmitted.)

In the case of diseases which are usually transmitted indirectly from man to man by means of some contaminated medium, such as water, it is conceivable that where conditions are adverse the chances of transmission occurring are so small that transmission actually does not occur, or occurs so infrequently that the cases are overlooked. A patient suffering from cholera, for instance, suddenly put down in the middle of London would, in all probability, not cause the occurrence of a single other case, but the same man put down in a village in the Punjab would, quite possibly, be the starting-point of a severe epidemic. Furthermore, that the disease is one which is spread by some contaminated medium is suggested by certain epidemiological observations, as, for example:

(i) the definite association with a certain kind of soil;

(ii) the greater incidence of the disease in rural areas, where sanitation is less satisfactory, and its association with general untidiness and accumulations of rubbish;

(iii) the greater incidence amongst the classes that are careless in their feeding habits;

(iv) the absence amongst breast-fed infants.

These epidemiological facts can, however, be explained on other grounds, and we have certain other observations which suggest very strongly that transmission is not such a simple matter as this. These are:

(i) the occurrence of the disease in a community has never been associated with any particular food or water-supply;

(ii) the thousands of infected persons that return to their homes on the Bihar plateau, or are imported into that district, from the highly infected areas in Assam, Bengal and Northern Bihar have failed to infect the rest of the indigenous population, although the conditions under which they live in the two areas are very similar;

(iii) the disease has failed to establish itself in Burma, where climatic conditions are almost identical with those of the indigenous areas;

(iv) the disease fails to spread in one crowded area in a town despite the presence of numerous imported cases, but from another area of the same town a succession of cases may be reported from one house.

These and numerous other facts that have been brought out in the study of the disease seem to be incompatible with the suggestion that the disease can be spread directly from man to man, or even indirectly by means of some inert medium, such as water or milk.

The main objection to this theory is that for infection to occur by this means it is essential that there should be a 'resistant' form of the parasite. Unlike most bacteria, this parasite is a very delicate organism and there is no chance whatsoever of the flagellate form with which we are familiar surviving for more than a few hours in any environment which it is likely to encounter in its journey from man to man during transmission, even if it were able to exist in the stomach of the person ingesting the contaminated material. As has already been pointed out, it does not survive in pure water or even milk for any length of time. Under special conditions the flagellate form appears to react to environment, and to 'round up' and become the Leishman-Donovan body; in this condition it is capable of resisting certain body fluids which would destroy the flagellate form, but even in this form it does not resist bacterial infection. Under other conditions, as, for example, in the presence of some special bacteria, it seems possible that the flagellate might be stimulated to encyst, and thereby avoid destruction by this and other bacteria.

A certain number of experiments in feeding animals on food and water taken from infected villages and houses have been carried out by Shortt (1923*b*) and others. These experiments were

entirely negative and, as such, are of no value; they would have be repeated many thousands of times with more susceptible animals than the ones used before acquiring any value as negative experiments.

Here, again, the case must be considered 'not proven'; but, in the opinion of the writer, the evidence is overwhelmingly against the possibility that the disease can, as a general rule, be spread without the aid of an intermediate host.

Under the heading of the contaminative theory must be considered the possibility that the disease is associated with some helminthic infection. The infecting organism might be ingested by the worm in the intestinal canal of the common host and transmitted by the ova to a new host, or, in the case of the hookworm, the leishmania might also be ingested by the larvæ from infected soil and thence carried directly to the new host.

The possible helminthic transmitters that have to be considered are Ankylostoma, Necator, Strongyloides, Ascaris, Trichuris and Oxyuris.

Ankylostoma, Necator and Strongyloides can be considered together. One of the strongest points against these three is that they have a geographical distribution which is much wider than that of kala-azar; they are much more prevalent in Ceylon, and also throughout the greater part of the Madras Presidency, than they are in Bengal and Assam. All three are also found in Burma. Furthermore, the incidence of kala-azar amongst pure town-dwellers, who show a very low hookworm infection rate, and amongst adult Anglo-Indians and Europeans, a class in which hookworm infection is rare, are points against the association of the two diseases.

The points in favcur of the hookworm are numerous; it is an infestation associated with the plains, a heavy rainfall, alluvial soil, an equable humid tropical climate, rural districts, and with site infection.

Knowles (1920) carried out a number of experiments with both varieties of hookworms found in Assam, and examined a large number of ova, larvæ and adults with entirely negative results. Christophers (1926) also reports negative results with Ankylostoma, Necator and Strongyloides.

Ascaris, Trichuris, and Oxyuris have a universal distribu-

tion; they are just as often encountered in cooler and drier climates and are not limited to the plains nor to alluvial soil. If any of these were the transmitter it is difficult to see why infection should tend to remain localised to one house or site.

The only points in favour of these worms is the fact that kala-azar is often associated with insanitary surroundings, and that the class which are least particular about their food are most frequently infected.

Lastly, under this heading, we must consider the possibility of the infecting organism being carried by some non-blood-sucking insect which does not act as a mere mechanical transmitter, but in which the parasite undergoes some development. From a protozoological point of view, this is a highly improbable hypothesis; both from this and from an epidemiological point of view many of the objections to the whole contamination theory of transmission are also applicable to this one; and none of the common insects that would be likely to be incriminated are confined to the endemic areas, although a more careful investigation into the insects of the endemic areas would have to be made before the theory could be discarded on this score alone.

The Theory of Insect Transmission.—Under this heading only the blood-sucking arthropods are considered. The most probable means by which infection could be transmitted is by the organism being taken in by the insect from the peripheral blood of the host and being transmitted to the next host by the bite of this insect. There are, however, other possibilities; these are—that infection is acquired by the insect from the peripheral blood of the infected man and is then transmitted to the second host by being crushed upon, or by fæcal contamination of the skin of the host, or by being ingested; or that the parasite is ingested by the insect in the latter's larval stage and then transmitted to man by a bite, or by being crushed upon and being rubbed into, or by contaminating with its fæces an abrasion in the skin of the new host.

If, then, the disease is passed from man to man by means of an intermediate host, what is the intermediate host?

This problem must be tackled from two points of view: first, by means of indirect, or circumstantial, evidence; and, secondly, by direct, or experimental, evidence. It is quite obvious that, until it

has been shown that the parasite is capable of development in the insect, and actual transmission has been reproduced under controlled conditions, the evidence for the final incrimination of that particular insect is not complete; but, on the other hand, it is equally obvious that it would be an impossible task to carry out experiments with all the insects found in the endemic areas, and also that when transmission has actually been effected by means of an insect, evidence would still have to be produced to show that this was the *usual* means by which the disease was transmitted. The best procedure to adopt, therefore, is to pick out the most likely insects by indirect methods, and to carry out experiments with these. This has, to a certain extent, been done, although there are still many possibilities to be explored.

Indirect Evidence.—The hypothetical transmitter must necessarily be present in all endemic areas, but is not necessarily absent from all non-endemic areas. A complete entomological survey of a number of endemic areas and a control survey of certain selected non-endemic areas would not necessarily solve the problem, but should lead to the narrowing down of the field of investigation; a complete entomological survey of any area is, however, a big undertaking. In the meanwhile, much valuable information as to the probable nature of the transmitter can be gathered by a careful study of the conditions which are apparently favourable to it.

It will not be possible to argue from the general to the particular, and to say that, because such and such conditions have been observed to be favourable to both one species of insect and to kala-azar transmission, therefore the insect is the transmitter of the disease; but it is obvious that before an insect can be incriminated, not only its geographical distribution but its general bionomics must undergo a critical examination in the light of our knowledge of the epidemiology of the disease.

It must be remembered that the causative parasite, when inside the body of the transmitting agent, will be protected from most external influences, but will still be affected by the temperature, and, in as far as it influences temperature, humidity; the former is probably the only factor that can directly influence the parasite, the other factors acting indirectly by their influence on the transmitting agent. Therefore, not only can an insect not be excluded from the list of possible transmitters, because it has been

shown to exist under temperature conditions which are unfavourable to transmission, but it cannot be excluded on the grounds that it flourishes under other conditions unfavourable to the transmission of kala-azar unless it can be shown that these other conditions are combined with the temperature conditions which are usually associated with kala-azar transmission. For example, the fact that bed-bugs are found in large numbers in hilly districts (where the temperature is outside the range of development of the parasite) does not prove that these insects do not transmit the disease, but their presence in the humid plains—of Burma, for instance, where temperature conditions closely simulate those of the endemic areas—provides a very strong argument that these insects are not capable of transmitting the disease.

It will be as well, first of all, to summarise the conditions which, according to an epidemiological study of the disease carried out by the writer (1926), must be favourable to the hypothetical transmitter, and then to consider the claims of the various arthropods, as has already been done in the case of a few helminths, to be included in the list of possible transmitters. Temperature conditions will be included in this summary, as it is possible, and indeed probable, that they play a dual part in determining the spread of the disease. These conditions are:

1. an altitude of less than 2,000 feet above sea-level;
2. a heavy annual rainfall, in the region of, or more than, 50 inches;
3. alluvial soil;
4. an equable humid tropical climate, as indicated by:

(*a*) a monthly mean maximum temperature that is always below 100° F.,

(*b*) a monthly mean minimum temperature that is always above 45° F.,

(*c*) an annual diurnal range that is less than 20° F.,

(*d*) an annual mean relative humidity of at least 70 per cent., and

(*e*) a relative humidity for at least three months of the year which seldom falls below 80 per cent. in the 24 hours;

5. a high subsoil water level, which is maintained as such throughout the year;
6. areas of abundant vegetation;
7. rural districts in general rather than towns;

8. in particular, old-established villages well sheltered by vegetation;

In towns, association with:

9. unprotected earth rather than paved courtyards and compounds;

10. vegetation in close proximity to the dwelling;

11. ground-floor residences;

12. thick-walled masonry houses rather than thin-walled bamboo and plaster huts;

13. accumulations of refuse and untidy conditions in the compounds, especially when these are connected with the keeping of chickens or ducks.

Furthermore, the arthropod must be one—

14. whose breeding ground is in some way connected with a definite site, in or around a dwelling, and most probably with the soil (as destruction of the dwelling still leaves the site infected);

15. which has a very short range of activity (less than 300 yards) and which, if a flying insect, has a very short range of flight and is very susceptible to air currents;

16. which does not readily establish itself on a new site, but which, under particularly favourable circumstances, is capable of a steady extension of range;

17. whose extension of range is checked by broad rivers, by the sea, by high ground (over 2,000 feet), and by unsuitable (non-alluvial) soil;

18. which does not ordinarily travel on man's person or in his belongings;

19. which is freely associated with human beings of both sexes, all ages, races, religions and castes, but which is on the whole more freely associated with poorer-class Anglo-Indians, Indian Christians and Muhammadans than with Hindus, and with children of the second and third quinquennial age periods; and, finally,

20. which is very prevalent—possibly more prevalent—during the monsoon months.

The scope of this book does not allow of the detailed consideration of the geographical distribution of all the possible transmitters, even if information on this point were complete. As, however, the incidence of the disease in the endemic areas is usually very high, amounting in some instances to at least 1 in 50 of the total popula-

tion of a district, it is obvious that the transmitting agent must be present in large numbers and that its presence is almost certain to have been recorded. We will, therefore, only exclude a suspected transmitter on grounds other than those of geographical distribution, except in cases where the distribution of the particular species is generally accepted as being a very wide one, or where the species has never been reported from the endemic areas.

Leeches have been suggested as possible transmitters; although they are not arthropods it will be convenient to consider their claims for inclusion as possible transmitters here. Their geographical distribution is not confined to kala-azar endemic areas, although it is possible that certain varieties have a more limited distribution. They are not limited to alluvial soil or to areas below 2,000 feet above sea-level; they are practically unknown in towns such as Calcutta; and they would not be associated with site or house infection.

On the other hand, they are associated with a humid tropical climate, rural conditions and abundant vegetation.

Of the blood-sucking arthropods, the ones that have to be considered are bed-bugs, fleas, lice, reduviid bugs, ticks and mites, Stomoxys and tabanids, mosquitoes, culicoides and sandflies.

The Bed-Bug.—*Cimex lectularius* is the variety commonly found in Assam and Bengal, but both this and *C. rotundatus*, the other recognised species, have a more or less universal distribution; they are not limited by altitude, rainfall, soil, temperature or humidity conditions. They are more likely to be associated with town than with rural conditions; they are not limited to ground-floor residences nor particularly associated with unprotected soil or vegetation. As they travel on the clothes and belongings of man, they can be transported considerable distances by rail, river or sea. In a comparative survey of an endemic and a non-endemic area in Calcutta, carried out by the writer (1925*b*), not only was it observed that the conditions for bed-bugs were equally excellent in both areas, but very large numbers were collected from the non-endemic area.

The points in favour of this insect being the transmitter are the house and site nature of the infection and its incidence amongst the poorer-class Anglo-Indians and Indian Christians, whose houses are always infested with bed-bugs. As these insects can live some months without food, the fact that a house remains infectious

although left empty for a considerable time is a point in their favour.

Fleas.—The common species in Bengal are *Xenopsylla cheopis*, *X. astia* and *Ctenocephalus canis*. These species are not limited to the endemic area; they are not as common in the humid alluvial plains of Bengal and Assam as in the drier districts in the north-west or in the hills. They are a personal insect, and could be carried on a man's person or in his baggage.

On the other hand, it is conceivable that they would give rise to a house or site infection, as their range is limited.

Lice.—These insects have a universal distribution and are far more prevalent in the hilly districts and in colder climates. They are not limited to humid climates. They are a personal parasite and would not necessarily give rise to house, and certainly not to site, infection. There does not seem to be a single epidemiological point in favour of these insects as possible transmitters.

Reduviid Bugs.—*Conorhinus rubrofasciatus* is known to be present in endemic areas; it is, however, not very common and seldom attacks man. It has a wide range of flight and would be unlikely to give rise to a house or site infection. On the other hand, its associations with rural conditions is a point in its favour.

Ticks.—Varieties of dog, fowl or cattle ticks are encountered, but in most instances they are even more common outside the endemic areas and are not limited by altitude or humidity. They would not give rise to a house or site infection. These varieties seldom bite man. On the other hand, most varieties would be more frequently encountered in rural areas, and persons living in close association with fowls would be most liable to be attacked by the fowl tick, *Argas persicus*.

Mites.—*Sarcoptes scabiei* is encountered in endemic areas, but its distribution is universal and, on the whole, is distinctly less common in these areas than in colder climates; there are no other mites that commonly attack man in these areas.

Stomoxys and Tabanids.—These flies are not limited to the endemic areas; they are not limited by altitude nor to the above recorded temperature and humidity conditions. They have a wide range of flight and do not remain associated with a house or site for any length of time. Only the former bites man. On the other hand, they will be found associated more with rural than with town conditions.

Mosquitoes.—The anophelines that are commonly encountered in Calcutta City are *A. subpictus* and *A. stephensi*; *A. fuliginosus*, *A. sinensis* and *A. barbirostris* are also found in the outskirts of the town. None of these species are limited to the kala-azar endemic areas. *A. subpictus*, at least, is not limited to the areas below 2,000 feet, and none of the species are limited to alluvial areas with a heavy rainfall and a high humidity.

Stegomyia argentea and *S. albopicta* are found in large numbers in Bengal, but they have an almost universal tropical distribution. The former is associated more with towns than rural areas.

The more important species of culicines that are found in and around Calcutta are *C. fatigans*, *C. vishnui*, *C. sitiens*, *Armigeris obturbans* and *Lutzia fuscana*. These again all have a wide geographical distribution and are not limited to the humid alluvial areas.

The range of flight of the mosquito is not a very limited one, and it would not remain associated with one site. Mosquitoes readily invade first-floor rooms, although few reach the top floor of a high building. A flight of 300 yards would present no difficulties to most mosquitoes, and their extension of range would probably not be impeded by a broad river.

On the other hand, most varieties are far more common in rural districts than in towns. In towns they are more associated with houses that are surrounded with vegetation and have dirty or untidily kept compounds

Culicoides.—Species that have been found in Calcutta are *C. macrostoma*, *oxystoma*, *brevimanus*, *quadrilobatus*, *gutifer*, *bimaculicosta*, *poeciloptera*. Some of these are blood feeders, which will occasionally bite man. None of them appear to be habitual human blood feeders. They are particularly favoured by the moderate humid climate of the endemic areas. They are much more prevalent in rural areas and are associated with abundant vegetation. They also occur in towns, but are associated with unpaved courtyards and untidily kept compounds containing much vegetation. They probably only invade ground-floor residences, as they are not strong fliers, but are not necessarily associated with masonry houses or the keeping of fowls and ducks. Their extension of range would be checked by broad rivers and the sea, and they would not ordinarily travel with man's belongings.

On the other hand, they are not limited to alluvial plains or to

altitudes less than 2,000 feet, although it is possible that certain species are found only within these areas.

Sandflies.—These insects have a very much wider distribution in India than has kala-azar. The species found in and around Calcutta are *Phlebotomus minutus*, *argentipes*, *papatasii* and *squamipleuris*. The first two are the common ones. *P. minutus* has a very wide distribution in India. The distribution of *P. argentipes* is much more limited, but this species is especially found in Bengal and Assam and feeds readily on man; it is only this species that need be considered in connection with kala-azar.

This sandfly is not observed above 2,000 feet, and its development is dependent on a high humidity and a moderate steady temperature; the high subsoil water level and alluvial soil help the maintenance of the necessary humidity throughout the drier months of the year.

Optimum conditions for the breeding of sandflies will be found in old-established villages surrounded by vegetation, especially around thick mud-walled huts where the soil has been well seasoned by animal pollution. In towns they will be found in dwellings which are surrounded with uncovered earth, especially when this is enriched with chicken droppings. Thick masonry walls will afford a better protection against the heat than a thin-walled bamboo hut; as the wall is dried by the heat of the sun it will continue to draw moisture from the moisture-holding alluvial soil, and will thus maintain a humid environment with a comparatively low and uniform temperature for any insect which hides within its crevices.

The range of flight of the sandfly is a short one, and it is seldom observed in any but ground-floor residences. Destruction of a hut by fire would probably leave the larval stages of the sandfly in the soil in and around the hut unharmed. Its extension of range will naturally be checked by broad rivers, the sea, high ground and unsuitable soil; it would not be carried on man's person or in his baggage. The conditions under which poorer-class Anglo-Indians, Muhammadans, and Indian Christians live form a suitable environment for this sandfly. The monsoon period, with its moderate temperature of low diurnal range, is the most suitable time of the year for its breeding.

There appear to be certain points in the epidemiology of the disease which favour many of the insects enumerated above as

possible transmitters, but in most of these cases there are other points which almost completely negative the suggestion. However, in the case of the sandfly *P. argentipes*, and the culicoides, the epidemiological points are almost all in favour of, and none against, these insects as possible transmitters of the disease.

Experimental Evidence.—The experiments that can be carried out in connection with this problem fall under four headings: experiments to show, firstly, that the parasite is capable of living and developing in the insect under investigation; secondly, that the parasite during its development reaches a position in the body of the insect from which one might reasonably expect it to find its way back to a new host; thirdly, that the parasite in the stage of development in which it exists in the insect is capable of causing infection in a new host; and, finally, that infection of a new host can actually be brought about by the agency of this insect under conditions which could conceivably be reproduced in nature. The last-named experiment is the only one that will provide actual proof, the first three experiments providing contributory evidence and constituting important steps in the process of investigation, for it is obvious that, unless these three facts can be demonstrated, there is little hope of success in the final experiment.

Experimental Animals.—A short digression on the subject of experimental animals will perhaps not be out of place here.

As there are obvious objections to experimenting with man, research workers have for some time been attempting to find an animal which is readily susceptible to kala-azar. Patton (1914) produced a fulminating type of the disease in a dog, but other workers in this country have found the dog disappointing; Knowles, Napier and Gupta (1923) produced only a scanty infection in one dog, and an infection both scanty and transitory in another out of a series of nine dogs, each of which received a large dose of material heavily infected with round forms of leishmania. The same workers produced a fulminant infection in a monkey (*M. rhesus*) by feeding it with post-mortem material, but report that as a rule only a transitory and very scanty infection occurs even when large amounts of morbid material are administered to this animal. Shortt (1923*c*) also produced an acute type of the disease in monkeys (*M. rhesus*). Workers have had uniformly disappointing results with rabbits and guinea pigs. Knowles, Napier and

Gupta (1923) had only negative results working with white rats and white mice of the Japanese variety, but the present writer has infected white mice—bred from a strain imported from England—with a fair degree of regularity by intraperitoneal inoculation of flagellate cultures, although here again the infection was always a scanty one and could often only be demonstrated by cultural methods. This has also been the experience of other workers (the Kala-azar Commission, 1926*a*). A number of attempts made by the writer to infect flying foxes were negative.

Recently, however, Young, Smyly and Brown (1924) pointed out that not only was the striped hamster, *Cricetulus griseus*, M.Edu., susceptible to the infection, but that in this animal the disease ran a course, chronic, progressive and usually terminating fatally, which was comparable to the course of the disease in man. As this animal is particularly easy to keep under laboratory conditions it should prove invaluable to the research worker. Melleney (1925) has made a very careful study of the histopathology of the disease, and concluded that, from an histological point of view, the hamster and man were strictly comparable. The fact that, by using this animal as the source of supply one is able to obtain absolutely fresh material at any stage of the infection, should provide an opportunity for a more thorough study of the pathology of the disease. For the purposes of experimental transmission this animal should also prove useful, but up to the present the Indian workers have had no opportunity of using it, as the first specimens have only recently arrived in this country.

Leeches.—No experimental evidence whatsoever has been brought forward in favour of these worms as possible transmitters. Mackie (1915) dissected 69 leeches, which had been fed on kala-azar patients and kept under observation for various periods, with entirely negative results, and the same experiment was carried out by Knowles, Napier and Gupta (1923), also with negative results; the latter used a selected patient showing a comparatively large number of parasites in the peripheral blood; they kept the leeches at 22°C. and dissected them at intervals up to 14 days. The leeches were caught in Assam in the former experiment, and in Bengal in the latter.

The Bed-Bug.—For nearly 20 years the bed-bug has been under suspicion as the possible transmitter of the parasite of kala-

azar. During this period a large number of workers have been engaged in an attempt to prove the case against this insect. Patton (1907, 1912 and 1922) has carried out a very large number of experiments with the bed-bug over a number of years; his experience may be summarised thus:

When bed-bugs are fed upon the heavily leishmania-infected blood of a kala-azar patient the parasite develops slowly in the gut of the bug into its flagellate phase, but if the bug takes another blood-meal within a short time the flagellates are destroyed; if, however, the second blood-meal is sufficiently delayed the parasites again round up, and a second blood-meal will not succeed in destroying them. Later, working with flagellate cultures, on which he fed the bed-bugs, following the method of Cornwall and LaFrenais (1916), and checking his results by cultural methods, he was able to show that, despite subsequent feeds on clean blood, the parasite was able to survive for at least 41 days in the gut of the bug, but that this only occurred in a small percentage of the bugs which received an infected feed—in the great majority of instances the parasites disappeared and could not even be detected by cultural methods. Adie (1921) discovered the so-called intra-cellular stage of the parasite in the gut wall of the bed-bug, *Cimex lectularius*, which had been fed on spleen-juice from a kala-azar patient. She observed flagellates attempting to penetrate the cells of the gut wall; when they had forced their way into the cell a sheath was formed round them. At this stage the flagellate was identical with Cornwall's (1916) thick tail stage. Later division occurred, and small new generations of flagellates burst into the lumen of the gut. Patton (1922) considered that this observation completed the evidence in favour of the bed-bug being the true intermediate host of the parasite of kala-azar. In view of the fact that the infection in the bug does not tend to pass forward to the biting parts of the insect, that the salivary glands have never been observed to be infected, and that Cornwall (1916) had failed to infect culture medium by means of the probosces of heavily infected bed-bugs which fed on the medium through a membrane, Patton concluded that infection of man occurs by the bug being squashed upon the skin and rubbed into an abrasion—possibly into the abrasion caused by the bite of the insect. Mackie, Das Gupta and Swaminath (1923) succeeded in infecting a mouse, by injecting into its peritoneal cavity

the contents of the gut of an infected bed-bug, and later Shortt and Swaminath (1924) infected another mouse in a similar way with the contents of the gut of a bug which had been fed on a kala-azar patient.

There are many points against the hypothesis that the bed-bug is the transmitter of kala-azar. Although the parasite will under certain circumstances develop therein, it is obvious that the gut of this insect does not form a suitable medium for its development; Patton, the greatest champion of the bed-bug theory, admits that development seldom occurs when one feeds the bugs on patients whose peripheral blood shows only 6 to 12 parasites per film, and that the patient's blood should show 20 to 100 parasites per film if development is to be expected; only a small percentage of cases in Bengal show the former numbers, and the latter is an exceedingly rare finding. Knowles, Napier and Gupta (1923) found parasites in only 12 per cent. of 442 films taken from 140 patients. Various workers have dissected many thousands of bed-bugs that have fed on unselected kala-azar patients; the number of infected bugs that have been found under these circumstances has been exceedingly low, certainly less than one per cent. The recent work on the sandfly—which will be referred to later—in which Napier and Smith (1926) and other workers have shown that practically every parasite that is ingested by the sandfly develops into a flagellate, shows up the shortcomings of the gut of the bug as a culture medium; the bug, taking a much larger blood-meal than the sandfly, must ingest some hundreds of parasites with each meal from an average patient.

Furthermore, it has been shown that the gut of the bug forms an equally suitable medium for the development of *Typanosoma lewisi* (Wenyon, 1912), *H. ctenocephali* (Shortt, 1923), and also for *Leishmania tropica* (Wenyon, 1911, and Patton, LaFrenais and Sundar Rao, 1921), yet the suggestion that the disease caused by this latter organism is transmitted by the bug has few supporters.

As already mentioned, no instance of salivary gland infection has been reported, in spite of the very large number of bugs that have been infected experimentally, and in no instance has it been noted that the infection was progressing towards the mouth parts of the insect, although Patton demonstrated the presence of viable forms in the rectum.

All attempts to infect animals by the agency of bed-bugs—with

the exception of the two instances recorded above, in which infection was caused by the injection of the gut contents of the bug into the peritoneal cavity of the animal, a method which could not conceivably be reproduced in nature—have failed. A large number of workers, Mackie (1915), Knowles, Napier and Das Gupta (1923), Shortt and Swaminath (1925), and others, have been engaged in this attempt over a number of years; the methods employed have been diverse, and include attempts at direct infection by feeding alternately on infected patients and experimental animals, feeding heavily, artificially infected bugs on animals after various intervals, crushing infected bugs on the scarified skin of animals and rubbing in the debris, rubbing in the fæces of infected bugs over scarified areas, subcutaneous injection of crushed bugs, and feeding of animals upon crushed bugs and their fæcal deposits. Patton and Sundar Rao allowed heavily infected bugs to feed freely on their arms; they did not become infected (Patton, 1922).

Many thousands of bugs which have been caught in the houses, and actually in the beds, of kala-azar patients have been dissected from time to time, but none of these has ever shown a leishmania infection.

The experimental work carried out with the bed-bug can be summarised as follows:

1. The parasite does develop in this insect, but not readily.
2. Infection of the salivary glands or mouth parts has not been observed, but the contents of the hind-gut have been shown to be infected.
3. The parasite in the stage in which it is present in the hind-gut of the bug is capable of causing infection in an animal when artificially injected.
4. Infection of an animal has not been produced through the agency of the bed-bug by any means that could conceivably be reproduced in nature.
5. A bed-bug naturally infected with leishmania has never been reported.

Fleas.—The evidence in favour of this insect mainly comes from the Mediterranean areas, and is based on experiments carried out with this virus. Basile (1911) reported that in fleas, *Ctenocephalus canis* and *Pulex irritans*, which had been fed on infected material, the flagellate form of the parasite developed; he also

showed that fleas fed on infected dogs developed a flagellate infection, and he claimed actually to have transmitted the infection to other dogs by means of these infected fleas. Other workers, including Gabbi (1911) and Da Silva (1914), failed to confirm his findings, and it seems very probable that he was misled by the fact that many fleas have a natural flagellate infection, *Herpetomonas ctenocephali*, for example. The gut of the flea not being an otherwise sterile medium, it seems improbable that the flagellate form of *Leishmania donovani* would develop readily therein. The workers in this country have had uniformly negative results, working with the species of flea that are observed in the endemic areas. Working with laboratory-bred fleas, *Ctenocephalus felis*, which had been fed on a dog showing a heavy leishmania infection of the peripheral blood, Patton (1914) failed to observe any development of the parasites and concluded that they died within 8 hours. Mackie (1915) failed to produce any infection in animals by injecting fleas which had been in contact with kala-azar patients.

Lice.—Patton (1912) dissected a number of lice, *Pediculus capitis* and *P. vestimenti*, which had been fed on kala-azar patients, and found no evidence of development of the parasite. Mackie (1915), working in Assam, examined a very large number of head and body lice taken from the bodies of kala-azar patients, and also injected subcutaneously the crushed bodies of some hundreds of lice into experimental animals, with uniformly negative results.

Triatoma.—A considerable amount of experimental work has been carried out with *Conorhinus* (*Triatoma*) *rubrofasciatus*; the results have been entirely negative. Patton (1912), using laboratory-bred bugs and feeding them on patients whose peripheral blood was rich in parasites, observed that the parasites remained unchanged for about 48 hours and then degenerated; he observed no development whatsoever, although the bugs ingested as many as 500 parasites. Negative results were also obtained by Donovan (1913) and Cornwall (1916). Knowles, Napier and Das Gupta (1923) carried out a large series of experiments with *Conorhinus rubrofasciatus*, feeding the insects at all stages of development, from nymph to adult, on infected peripheral blood, on spleen puncture material mixed with citrated blood, and on spleen pulp from patients who had died of kala-azar, keeping the insects at various temperatures and dissecting them after various intervals; the results were all negative.

Ticks.—Patton (1922) demonstrated that no development occurred in the tick *Ornithodorus savignyi* after feeding on kala-azar patients, and Donovan (1913) also reported negative results with this tick.

Mites.—Mackie, Gupta and Swaminath (1923) examined a number of specimens of *Acarus scabiei*, taken from kala-azar patients, with negative results.

Mosquitoes.—Franchini (1912), working with anopheles mosquitoes from Ferrara, found that the ingested parasite lived for 48 hours; he appeared to attach some importance to this finding, but from his own description of the experiment it seems obvious that they were undergoing degeneration.

Patton (1907) failed to observe any development in three species, *Culex fatigans*, *Anopheles stephensi* and *Stegomyia sugens*, and Mackie (1915) failed to produce infection in experimental animals by the injection of the crushed remains of a large number of mosquitoes, culex and anopheles. Knowles, Napier and Das Gupta (1923) failed to observe any developmental forms in a number of stegomyia fed on infected spleen emulsion in citrated blood.

Culicoides.—The workers at the Calcutta School of Tropical Medicine (Napier, 1925) found that most species encountered in Calcutta did not appear to feed readily on man, but their work on the culicoides was interrupted by their discovery that flagellation readily occurred in the sandfly *P. argentipes*, which necessitated their concentrating on this insect. The Kala-azar Commission (1925) reported that they were able to persuade *C. macrostoma* to feed on kala-azar patients, but that no development occurred in this insect. The Calcutta workers failed to persuade this species to feed on man, and they were of the opinion that it was a plant-feeding species.

The Sandfly.—The first observations on the development of the parasite in the sandfly were made by the workers at the Calcutta School of Tropical Medicine in September, 1924 (Knowles, Napier and Smith, 1924). Eleven batches of laboratory-bred female *Phlebotomus argentipes* were fed on unselected kala-azar patients in hospital under the present writer's charge; on dissection, on the third to fifth days, it was observed that 25 out of 56 flies had a herpetomonad infection in the mid-gut. Forty-six flies, bred under the same circumstances, were fed on the peripheral blood of persons

not suffering from kala-azar, and in no case was a herpetomonad infection observed. It was, therefore, concluded by these workers that *L. donovani* passes into its flagellate stage in the gut of *P. argentipes* under suitable conditions of atmospheric temperature and humidity. Early in the next year the Kala-azar Commission (1926 *c*, *d* and *e*) published three papers confirming these findings.

Up to this point the difficulty that had confronted all the workers had been the fact that the sandflies did not appear to take a second blood-meal as a general rule. If this were the case it was necessary to postulate that transmission took place by the passing of the infection to the next generation. Napier and Smith (1926) concluded that the most likely way for this to occur was by the larvæ of the next generation devouring the dead adults; they therefore fed a number of larvæ entirely on the bodies of dead infected flies, but in no instance were they able to breed a congenitally infected fly. The same difficulty presenting itself to the Kala-azar Commission (1926*f*), they attempted to produce infection in the unfed fly by feeding the larvæ on cultures of *L. donovani*, also with negative results. Summing up their experience of feeding sandflies on kala-azar patients over a period of nearly one year, Napier and Smith (1926) concluded that every viable parasite ingested caused a flagellate infection of the gut of the fly if the temperature conditions were suitable, that the monsoon was the most suitable period for development, and that during the coldest months practically no development took place; they also reported that, although it was difficult to persuade sandflies bred at the ordinary laboratory temperature during the months of November to March to survive the first oviposition and feed a second time, during the month of August, when monsoon conditions had been established for about one month, there was little difficulty in getting the flies to feed a second or even a third time.

The 'monsoon incubator', which was devised to reproduce monsoon conditions during the cold weather months of 1924-25, was a failure—the temperature therein rising to 100° F. one night led to the destruction of the whole of the sandfly breeding stock—and had to be abandoned.

Meanwhile the Kala-azar Commission (1926*g*) was making rapid progress towards what appeared to be the solution of the problem; they demonstrated that by keeping artifically specimens

of *Phlebotomus argentipes* through all the stages of their development at the monsoon temperature of 83° F., a fly of good stamina, which would feed readily a second, third and fourth time, could be produced, even in the cold weather months. By careful section cutting they were able to demonstrate a massive infection of the pharynx and a definite infection of the buccal cavity of sandflies, on the sixth and seventh days after they had fed on kala-azar patients and had fed once during the interval on the same patient or on a mouse; they also demonstrated a pharyngeal infection in fifth-day flies which had fed once on a kala-azar patient. Finally, they (Kala-azar Commission 1926*j*) demonstrated a massive infection of the buccal cavity extending distally to the salivary pump.

By this time the position with regard to the transmission problem was that, under suitable conditions of temperature and humidity, every viable form of the parasite taken in by the fly developed into a flagellate, that rapid multiplication occurred in the gut of the fly, that the infection passed forward so that if the fly took a second meal and survived until the seventh day the buccal cavity eventually became infected, and that if this fly fed a third time it was practically impossible for contamination of the wound by flagellates not to occur.

The Kala-azar Commission (1926), in a study of the life history of the parasite in the sandfly, showed that all the various types that had been observed in the culture tubes were to be found in the sandfly, with the exception of the so-called cystic stage. They pointed out that because they had not observed the cystic stage it did not mean that this stage did not occur in the sandfly.

Lloyd, Napier and Smith (1925) showed that in nature this sandfly fed readily on man, and the Kala-azar Commission (1926*k*) report the finding in nature of a specimen infected with herpetomonad forms indistinguishable from leishmania.

At the time of writing many experiments aimed at the transmission of the infection to animals have been undertaken by the workers at the Calcutta School of Tropical Medicine, and by the Kala-azar Commission, but up to the present no success has been announced; it is, however, too early to state that these experiments have failed.

Napier and Smith (Napier, 1926) carried out a small series of parallel feeding experiments with *P. papatasii* and *P. argentipes*;

they did not observe any development in the former species, whereas in the latter development was observed in the usual percentage of flies.

These experiments were repeated during the monsoon months of 1926, and development of the typical leishmania flagellate was observed in 43 out of 102 specimens of *P. argentipes* and in 2 out of 101 specimens of *P. papatasii* fed on the same kala-azar patients (Napier and Smith, 1927).

Following the lead of the workers in this country, the workers in China investigated the sandflies of their endemic areas. They found three species, *Phlebotomus major*, var. *chinensis* and two other species which have not yet been identified; these they have called provisionally 'B' and 'C'.[1] Development of the parasite was observed in *Phlebotomus major* in 29 flies out of 34 fed on kala-azar-infected hamsters, but none developed in the flies fed on kala-azar patients; no development was observed in *Phlebotomus 'B'*, but in *Phlebotomus 'C'* development was observed in 7 out of 373 specimens fed on kala-azar-infected hamsters (Young and Hertig, 1926).

A SUMMARY OF CONCLUSIONS REGARDING THE KALA-AZAR TRANSMISSION PROBLEM

The weight of evidence appears to point to the fact that the disease is transmitted from man to man, that is to say, that infected man is an essential link in the transmission chain. There is little evidence to show that the causative parasite leaves the infected man by any route other than that of the peripheral blood. It is most probable that the route by which the parasite enters the new host is through the skin, although other means of entry are not entirely excluded. The evidence appears to be against the disease being transmitted directly from man to man, and it seems probable that it is transmitted by means of an intermediate host. This intermediate host is probably a blood-sucking insect. Of the various insects that are considered as possible transmitters, indirect evidence singles out the culicoides and the phlebotomi, and, in that its known distribution bears some relationship to that of kala-azar,

[1] According to Patton and Hindle (1926), *Phlebotomus 'B'* is a new variety of *P. perturbans* de Meijere and *Phlebotomus 'C'* is a variety of *P. sergenti* Parrot described by Newstead.

P. argentipes falls under the greatest suspicion. The bed-bug and the sandfly are the only two insects that have any experimental evidence in their favour, but in view of the fact that the gut of *P. argentipes* appears to constitute a medium a hundred times more favourable for the growth of the parasite than does the gut of the bug, this insect is more likely to be the true intermediate host of the parasite. As the parasitic infection in the intestinal tract of the sandfly shows no tendency to die out, but rather to increase, to spread anteriorly and eventually to involve the buccal cavity, the probability is that infection of man occurs through the proboscis of this insect during the process of blood-sucking. At the time of writing the actual experimental proof that the disease can be transmitted in this way is wanting.

THE IMPORTANCE OF THE SECONDARY FACTOR IN KALA-AZAR TRANSMISSION

The patient suffering from kala-azar who receives no specific treatment usually lives for at least two years; during the whole of this period parasites are present in the peripheral blood. In the villages in the kala-azar endemic areas *Phlebotomus argentipes* is present in large numbers for a greater part of the year. At least 20 per cent. of the flies that feed on a kala-azar patient become infected, and for at least three months of the year the conditions are favourable for the long life of the fly, and, consequently, for the full development of the parasite in the fly. Therefore, if this sandfly is the transmitter of the disease, one would expect the introduction of a patient suffering from kala-azar into a potentially endemic area to be followed very rapidly by the infection of the whole community. It is only very rarely that this occurs; usually only a small percentage of the population become infected at a time, although over a period of years a very high percentage may eventually become infected. It is thus obvious that there is a brake somewhere, and it seems probable that the body-resistance of the population to the invasion of micro-organisms constitutes this brake.

This resistance may be local or general. We know that in order to infect a mouse it is necessary to introduce a comparatively large dose of flagellates. The dose of flagellates that a sandfly could conceivably introduce is very small indeed, so that in order

to infect a healthy man repeated doses may be necessary. On the other hand, man in a state of health may be immune, and it may be the introduction of the secondary factor, in the form of some intercurrent infection—typhoid or malaria, for example—which allows invasion to occur. Again, it is possible that the parasite obtains a footing at the site of injection without much difficulty, but that subsequent systemic invasion is dependent on the secondary factor. Or, as a third possibility, a general systemic infection may occur, which would, in the ordinary course of events, be transitory, but the morbidity of this is determined by the secondary factor.

Although all these suggestions are to a large extent theoretical, they have been suggested by actual experience. The majority of patients suffering from kala-azar give a history of an onset which simulates either typhoid or malaria. It is not an uncommon experience for a patient suffering from bacteriologically-proven typhoid to show the first symptoms of kala-azar during the convalescent period (Shanks and Khan, 1926); it has been shown that the agglutinating power of the blood for *B. typhosus* is very rapidly lost in such cases. Furthermore, epidemics of other diseases are repeatedly followed by a great increase in the incidence of kala-azar in the local population.

The writer has had personal experience of a number of cases in which the initial attack of fever—during which attack leishmania was demonstrated in the peripheral blood—was not followed by any further symptoms, although no specific treatment was administered, and recently he has seen in Bengal a large number of cases of dermal leishmaniasis—a condition which has been looked upon previously only as a sequel to kala-azar—in which there has been no history of kala-azar and in which the patient has certainly not been treated for the disease; both these facts add strength to the suggestion—made some years ago by the present writer (1923)—that in man living in suitable surroundings in the endemic areas leishmaniasis is a comparatively common infection, which may be mild—or symptomless—and transitory, or may, under special circumstances, become a definite and progressive disease.

In the laboratory-infected monkey this transitory infection appears to be the rule, although occasionally a fulminant infection is produced.

Phlebotomus Argentipes

This is a medium-sized sandfly, about 2·3 to 2·8 millimetres long; it is dark brown in colour; the dorsum of the thorax is black and the

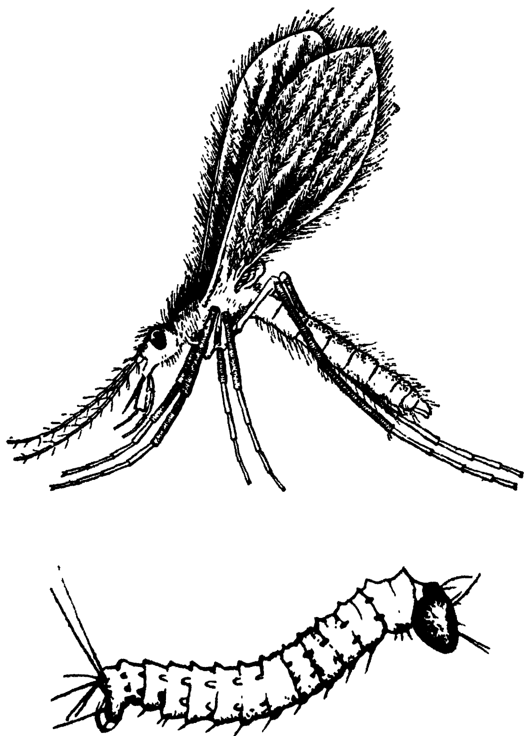


Figure II

Phlebotomus argentipes, adult (× 20), larva and ovum (× 60).

sides are light yellow; the wings are rather broader than those of most species; and the tarsi are white. A detailed description, including the distinguishing characteristics of the species, is given by Sinton (1923) and by the Kala-azar Commission (1926*h*).

GEOGRAPHICAL DISTRIBUTION

According to Sinton (1924 and 1925) this species has not been found outside India and Ceylon. He reports that it has been found in Poona, Kamptee, Lucknow, Pusa, Purneah, Rajmahal, Asansol, Madhupore, Calcutta, Port Canning, Puri, Madras, Pallode, Travancore, Maddathorii and Ceylon.

Recently, Barraud (1926) reported the finding of large numbers in the kala-azar endemic areas in Madras Town; the Kala-azar Commission have found large numbers in the endemic areas in Assam; the writer and other workers at the Calcutta School of Tropical Medicine have found large numbers in the kala-azar-infected villages around Calcutta, as well as in the city itself. The writer has found this species in Purulia, Nagpur, Bhusawal and Bombay, where it has also been reported by McCombie Young (1927).

There are certain details in the bionomics of this sandfly which may have a special bearing on the kala-azar transmission problem (Napier and Smith, 1926*a*).

BIONOMICS

Habitat.—The flies are found in cowsheds, human sleeping quarters, and occasionally in certain other situations, such as fowl-houses. They are found in the largest numbers in the cattle sheds well protected from wind currents with masonry or thick mud walls reaching up to the roof, and with ill-paved or unpaved floors. They are found in smaller numbers in the ground-floor rooms of masonry houses when these are damp, dark and ill-ventilated, have a paved floor which is broken in places, and a small window which opens out on to a courtyard where fowls, goats or ducks are kept. They are also found in the typical living room in the huts in the rural areas of Assam and Bengal.

The larvæ have been found in a number of situations, in rat holes in a house, in the corner of a living room where the cement floor was broken and where earth and rubbish had collected, in material collected from the floor of an outhouse in which goats had been kept, and very frequently in the material collected from the floors of fowl-houses. They appear to thrive in any earth which contains a sufficient admixture of nitrogenous matter and is not too dry. Although the writer has only found specimens in the

above-mentioned places and other workers have not reported the finding of the larvæ of this species, it seems probable that they are to be found in the open in places where they are protected from the sun, from desiccation during the hot dry weather, and from excessive moisture during the rainy season.

These observations probably explain why kala-azar is confined to moisture-holding alluvial soils, why it is associated with unprotected earth and vegetation, the latter association being a partial correlation, and why it is associated with unsanitary surroundings, especially when these are due to the presence of fowls or ducks.

Prevalence.—The investigators at the School of Tropical Medicine have collected these sandflies from places in Calcutta and its environs at all seasons of the year over a period of two years. On the other hand, workers in Assam reported last year that they were compelled to abandon their work for a period of about two months, as during the hottest months of the year wild flies were not obtainable. In Calcutta the largest numbers are observed during the latter half of the monsoon; the sandfly curve begins to rise about a month after monsoon conditions have become well-established (the latter half of July), and remains high until the beginning of the cold weather (November). During the cold weather months (December and January) there is a very marked fall in the number of flies, and during this period they are practically only found in cattle sheds; this is apparently also true of the flies in Assam (Kala-azar Commission, 1926*c*). During the period when the temperature is beginning to rise larger numbers are found, but there is again a decided falling off during the hot, dry months preceding the monsoon. In the laboratory, breeding can be continued throughout the whole year at room temperature, but it slows down very much during the cold weather months.

The Effect of Atmospheric Conditions on the Stamina of the Flies.—During the cold weather months wild flies, which when caught have usually fed, can seldom be induced to take a second feed, and flies bred in the laboratory during this period are also of poor stamina; if they feed once they usually die during or immediately after oviposition. This is also true of flies bred during the hot dry season. In a normal year in Bengal, when the temperature begins to rise towards the end of February, the humidity begins to fall, so that it is only during the monsoon season that conditions

are really favourable. During these months the wet-bulb temperature seldom falls below 80° and the atmospheric humidity is seldom, if ever, below 80 per cent. and the diurnal range of temperature is extremely low; within a building this is often less than 1° Fahrenheit. This is shown in the two accompanying charts. Chart V is a reproduction of a thermograph chart recording the temperature in a corner of the writer's laboratory in Calcutta during one week in August; and Chart VI is prepared from thermograph readings taken from July 21st to August 29th in the same place.

These are the conditions which have been found most favourable for the breeding of flies of good stamina in the laboratory, and in nature during this season, as already stated, the largest numbers of flies are observed.

The importance of these conditions is shown both in the kala-azar onset curve—which, as already shown, commences to rise sharply in the month of September—and in the distribution of the disease in India; the disease has shown no tendency to spread from its focus in Bengal into areas where these favourable conditions are not maintained for some months during the year. The absence of the disease from such places as Bombay, where climatic conditions are very similar and where *P. argentipes* is found, may be due to the fact that the mean temperature during the three monsoon months is about 3°F. lower than it is in the endemic areas in Bengal and Assam.

Feeding.—The adult female sucks blood and is a pure blood-feeder. In the males we have only found the remains of the larval feed. The female appears to be a selective feeder, and almost always to choose either man or the cow. Neither avian nor reptilian blood was ever found in wild *Phlebotomus argentipes*, and in nearly every instance the blood has been shown to be bovine or human (Lloyd, Napier and Smith, 1925). Where there was a choice between human and bovine blood, the latter was usually chosen; but where the choice lay between fowls', ducks', dogs', cats', goats' or human blood, human blood was chosen.

This observation suggests a possible prophylactic measure where it is impossible to keep the inhabitation entirely sandfly-free.

Laboratory-bred flies will, however, feed readily on most laboratory animals; they seem to feed best if kept for at least 24 hours before being placed to feed; they then feed at intervals of 60 to 72

CHART V

Thermograph reading taken in the corner of a laboratory in Calcutta for one week during August.

hours, that is, on the 2nd, 4th or 5th, 7th or 8th, 9th to 11th, and 12th to 14th day of their lives.

Flight.—They have a very limited flight. When disturbed on a wall they do not fly a distance of more than a few inches; they give the impression of hopping rather than flying. They are not found in unused rooms or in any place far from their food supply. Large numbers are often found in a cattle shed adjoining a house, without any being found in the house itself. On one occasion larvæ were recovered from a hole in the corner of one room in a row of servants' godowns in the compound of a house in Calcutta; 94 flies were caught from this room during three successive visits; one fly was found in the adjoining room, but none were ever caught from the other rooms of the group, although the rooms were all used as sleeping quarters and the doors of the rooms were less than six feet apart. They are not found in the upper rooms of houses.

This may account for the extremely localised nature of the infection in kala-azar, the fact that ground floor residents only are attacked and that the removal of a coolie line a distance of 300 yards effectually checks the spread of the infection.

HISTORY OF THE FIRST INCRIMINATION OF THE SANDFLY

In view of the position at present held by the sandfly, *P. argentipes*, in relation to the problem of the mode of transmission of kala-azar in India, it will perhaps not be out of place to recount the events which led to the first incrimination of this insect.

During the years 1921 and 1922 the writer, who was in charge of the Kala-azar Research Department at the Calcutta School of Tropical Medicine, had been carrying out investigations with the object of finding the means by which the disease was transmitted. Up to this point his efforts, as far as the transmission problem was concerned, had been almost entirely sterile, except that it might be claimed that here and there a *cul-de-sac* had been explored. At an informal meeting between Majors Acton and Knowles, professors at the Calcutta School of Tropical Medicine, and the writer, it was decided that further investigation into the epidemiology was necessary, more especially with reference to the month of onset of the disease, and this matter was taken up by the writer. Careful notes had been made on all the cases of kala-azar seen at the well-

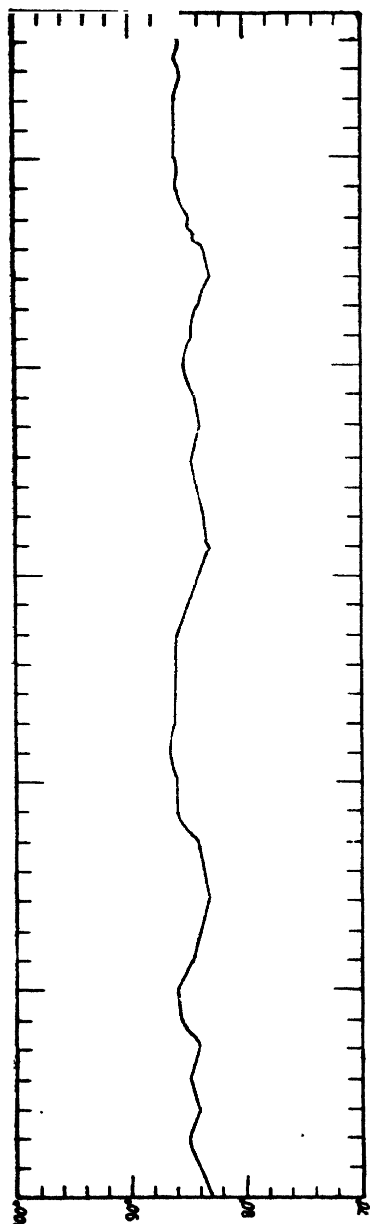


CHART VI

Daily record of temperature in a laboratory in Calcutta from July 21st to August 29th. The variation is within 3°F.

attended out-patient department which had been started at the school, as well as on the cases at the hospital attached to this institution, so that a considerable amount of material was ready to hand for this investigation. Two important facts came to light: one was that the kala-azar onset curve commenced to rise in September, the other was with regard to the distribution of the disease in Calcutta; it was observed that, although imported cases came from all parts of the town, of the patients that had acquired the disease in Calcutta about 50 per cent. came from one district and from another district practically none (Napier, 1925*b*). A general survey, which aimed at the observation of the conditions of living in the endemic area, using the kala-azar-free area as a control, was undertaken by the writer during the monsoon and the cold weather of 1923; every attempt was made to obtain expert entomological assistance in order that an entomological survey might be carried out in July, August and September, during which months, it was assumed, the greatest number of infections occurred, but without success; Dr. Strickland, the professor of entomology of the school, was engaged in Assam at this time on malaria investigations, and the only insect collector whom he could spare proved very unsatisfactory. Thus the most favourable time of that year was missed, and the general survey had to be completed without entomological assistance.

Early next year the writer went on leave to Europe for a few months, and his work was taken over temporarily by Major Knowles; the latter, with the assistance of Major Acton, made another determined effort to obtain entomological assistance in order that the favourable time of the next year might not slip by. Major Acton appealed to the Indian Research Fund Association, who placed Military Assistant Surgeon R. O. A. Smith at the disposal of the workers of the school. In accordance with the previously formed plan, he commenced an entomological survey of the two Calcutta areas, but it was decided that, in view of the fact that the observations of the writer on the conditions under which kala-azar existed in Calcutta communicated to Major Acton had strengthened the already firmly rooted opinion in the mind of the latter that some species of sandfly was the transmitter of the disease, experimental work during the first season should be confined to two genera, phlebotomus and culicoides. The writer returned to duty in

July, just three weeks after this investigation had commenced, and from this time forward he worked in collaboration with Major Knowles, although Major Acton, who was then acting Director of the school, continued to help with his advice.

By the end of the second month of this investigation certain quite definite observations had been made; it was found that *P. squamipleuris* was rare, that *P. minutus* was common in both areas in Calcutta, and that *P. argentipes* was confined almost entirely to the endemic areas, the largest numbers being found in houses from which cases were reported and in the outhouses in the compounds of these houses; and that culicoides of all varieties were found in the endemic area. It was felt that this finding of *P. argentipes* in large numbers in the infected houses in the endemic area was very suggestive, in view of the fact that it had been pointed out by Major Sinton—in answer to an enquiry on the subject of sandflies of the kala-azar endemic areas—that *P. argentipes* appeared to have a distribution in India corresponding with that of kala-azar, although up to that time it had not been reported from Assam or Madras City (Knowles, Napier and Das Gupta, 1923). Laboratory breeding of the two species of sandflies, *P. argentipes* and *P. minutus*, and of a number of species of culicoides was undertaken by Dr. Smith and feeding experiments were carried out on the arms of kala-azar patients. The results obtained with *P. minutus* and the culicoides were entirely negative but it was found that *P. argentipes* fed readily, and that *in a large percentage of specimens the parasite developed into the typical herpetomonad form within 72 hours* (Knowles, Napier and Smith 1924). Control experiments were carried out and the results immediately communicated to the Kala-azar Commission, then working in Assam. From this time onwards both enquiries concentrated their efforts almost entirely on this sandfly; the results of their investigations have been recorded above.

In view of the weight of the evidence against this sandfly as the transmitter of kala-azar, it is rather difficult to understand how it has escaped incrimination at an earlier date. As the writer is guilty of the statement (Napier and Muir, 1923), 'From an epidemiological point of view, it can hardly be said that the facts are as much in favour of the sandfly as of the first two mentioned insects (i.e. the bed-bug and the flea)', he must first of all attempt to excuse him-

self. What he had in his mind when he wrote this was the fact that not one in a hundred of the residents of Calcutta knows that sandflies exist in the town, whereas all the inhabitants of most places in Northern India and in Mesopotamia, where the disease is not endemic, are at certain times of the year painfully aware of the presence of these pests, as such. But the fact that he had included it as one of his four 'suspects' at least suggests that he considered that it was an insect worthy of further investigation. Of the earlier investigators, Mackie (1915) considered that the sandfly was worthy of further investigation, but as he only reported the finding in Assam of *P. minutus*, a variety which lives only on the wall lizard and seldom bites man, there was little encouragement to follow this suggestion. Patton (1922) dismissed the sandfly on the ground that he was never able to find any specimens, other than *P. minutus*, in the endemic areas in Madras City. Awati (1922) in his entomological survey of Assam did not report *P. argentipes*, nor was any mention made of this species by Mackie, Das Gupta and Swaminath (1923), or by Shortt (1923*b*) in his investigations prior to the formation of the Kala-azar Commission. Furthermore, little was known of its breeding habits, and, prior to the work of Waterston (1921), nobody had succeeded in breeding members of this genus in captivity. This is rather surprising, as the problem presents few difficulties, in Bengal at least, if attempted at the right time of the year.

The Probable Life Cycle of *L. donovani* and the Probable Mechanism of the Infection of Man.—The parasites are present in the peripheral blood of a very high percentage of cases—if not of every case—of kala-azar. They are to be found lying in the polymorphonuclear, in the large mononuclear and transitional leucocytes; the parasites in the first-named do not stain as clearly as do the others, and it seems possible that they are being phagocytosed; it is, however, certain that many of these are still viable. In the peripheral blood the parasite is present in the pre-flagellate or 'round' stage—the Leishman-Donovan body. They may be present in large numbers, so that little difficulty is experienced in finding them in a blood smear, but as a rule they are present in very small numbers, one or two blood films having to be searched before a parasite can by found. If one or more of these parasites be taken into the mid-gut of a sandfly in a blood-meal, development commences, the round form becomes a flagellate

form and active division occurs. By the third or fourth day there is a massive infection of the mid-gut of the fly; at the anterior end the whole lumen of the gut is blocked by a solid plug of flagellates; these are mostly the stouter dividing forms of flagellate, arranged in palisade formation two or three deep around the lining of the mid-gut, but the slender free-swimming forms are also present. By this time the greatest aggregation of parasites is in the anterior part of the mid-gut around the proventricular opening, only a few parasites having passed through the œsophagus. The fly will now take a second blood-meal; this second blood-meal does not appear to have any deleterious effect on the flagellates, but, on the other hand, allows the life of the fly to be prolonged, so that by the seventh day the massive flagellate infection has progressed beyond the œsophagus into the pharynx and commenced to invade the buccal cavity. Active forms have been observed as far forward as the base of the proboscis, so that when the fly takes another blood-meal it is almost impossible for it to avoid inoculating a certain number of flagellates.

We must now leave the realms of observed occurrence for those of probability.

It seems probable that the following is the sequence of events:

A certain number of these actively mobile slender forms—which are probably the infective forms—are injected, together with the salivary material, into the layer of the skin wherein lie the capillary tufts from which the fly obtains blood. The flagellate finds its way into a cell in this situation and becomes a round form again. Here it remains, undergoing slow multiplication, but does not at first invade the rest of the body. Then some change occurs, possibly the body resistance is lowered by some other febrile infection, and invasion of the whole system occurs; or it is possible that this invasion itself is manifested by a febrile attack. There is the usual struggle between the body and the invading organism, and one of three things occurs.

Firstly, the parasite may win completely and invade all the tissues of the body; the patient may be considered to have got kala-azar, and symptoms will show themselves after an interval, varying in different individuals; the parasite will now appear in the peripheral blood, and, if another sandfly feeds on the patients' peripheral blood, the cycle will begin again.

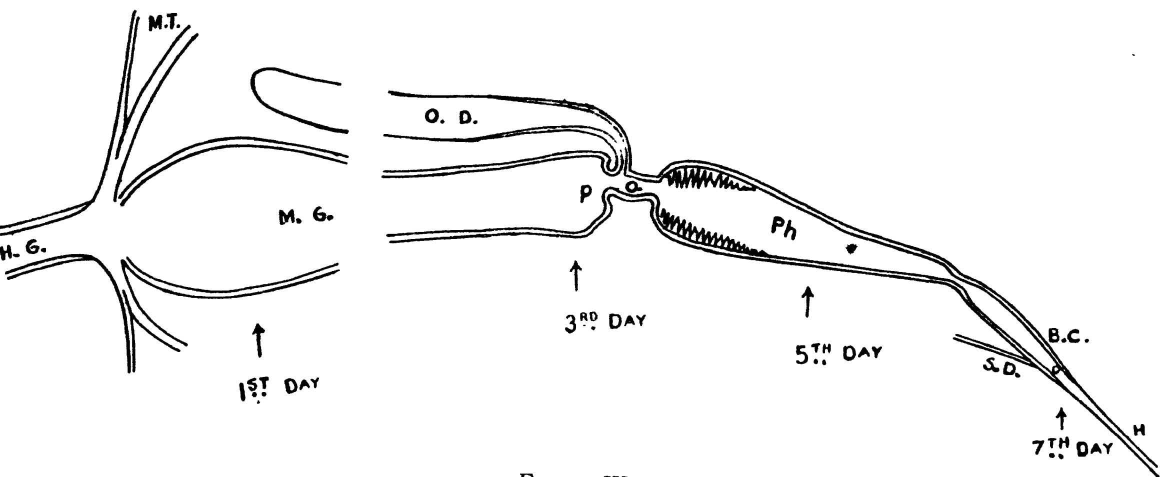


FIGURE III

Diagram of intestinal tract of the sandfly. The arrows indicate the points to which the flagellate infection reaches on the various days after the fly has fed on a kala-azar patient.

H.G.	Hind-gut.	O.	Œsophagus.
M.G.	Mid-gut.	Ph.	Pharynx.
M.T.	Malpighian tubules.	S.D.	Salivary ducts.
O.D.	Œsophageal diverticulum.	B.C.	Buccal cavity.
P.	Proventricular fold.	H.	Hypostome.

Secondly, there may be a complete victory for the body—all the parasites being killed.

Or, thirdly, the parasites, having invaded the whole body, may now be driven back to the hyper-susceptible, or protected, area in the skin, the body resistance being able to deal with all the parasites in the other tissues, but those in the skin being still able to hold their own. The parasites will multiply slowly in this situation, and eventually the pathological condition of dermal leishmaniasis[1] will be established, at first white patches and later small nodules appearing in the skin. As parasites occasionally appear in the peripheral blood, it seems possible that in this condition the patient acts as a 'carrier'.

Figure III gives a diagramatic representation of the alimentary tract of the sandfly, the points to which the flagellate infection reaches on the various days being indicated.

[1] Although this condition is more commonly observed in treated patients, a certain number of cases have been noted in which the patient, although living in an endemic area, gave no history of having had a definite attack of kala-azar, and had certainly not been treated for the disease; it seems possible that these patients have had an abortive attack of the disease which was not recognised as such.

CHAPTER III

MORBID ANATOMY AND HISTOPATHOLOGY

General nature of pathological changes—Spleen—Liver—Bone marrow—Lymphatic glands—Intestinal tract—Supra-renals—Thyroid—Kidneys—Heart—Testes—Post-kala-azar dermal leishmaniasis.

CHRISTOPHERS (1904) was one of the earliest workers to give an accurate description of the pathology of kala-azar. He pointed out that the parasites invaded almost every organ and tissue in the body; that they entered and multiplied in the endothelial cells of the capillaries; that with the multiplication of the parasites these enlarged and, eventually, burst; that the phagocytic cells of the blood and tissues also took up the parasites; and that in these phagocytic cells the Leishman-Donovan bodies were not destroyed, but remained viable and multiplied.

Since the appearance of this paper a number of contributions have been made to our knowledge of the pathology of the disease. The lesions in infantile kala-azar have been described by workers in Europe; the pathology of canine leishmaniasis and that of experimentally-infected monkeys (Shortt, 1923*c*) has been investigated; and recently a very detailed description of the histopathology at various stages of invasion of the parasite in the hamster has been contributed by Meleney (1925).

Shortt and Brahmachari (1925) reported the histological findings in a case of dermal leishmaniasis, and Acton and Napier (1927) have described the findings in a number of cases in various stages of this disease.

GENERAL NATURE OF PATHOLOGICAL CHANGES

It is obvious that the lesions in the various forms of generalised leishmaniasis are all very much of the same nature, and that any differences that exist are due to individual variation in the hosts, or to the fact that the observations have been made at different stages in the process of invasion of the parasite. The steady progress of the invasion was very well demonstrated by Meleney, who examined

hamsters which had been infected with leishmania for various periods, from 11 to 455 days.

In every case the parasites are present in large numbers in the spleen, liver and bone marrow. The lymphatic glands are also usually involved. A lesser degree of infection is observed in the other organs and tissues of the body, with the exception of the nervous system ; the presence of the parasite in the cerebro-spinal fluid has been reported. It is in the endothelial cells of the capillaries and in the macrophages in the connective tissue that the largest number of Leishman-Donovan bodies are seen. The parenchymatous cells in the liver are usually invaded at a later stage of the infection, and the invasion of the glandular tissue of the supra-renal gland has been shown both in man and in the hamster; otherwise the parenchymatous cells of the organs appear to avoid infection.

Meleney claims that there is a specific tissue reaction in mammalian leishmaniasis. The cell involved is the large mononuclear phagocyte, the macrophage. The reaction is most marked in the reticular tissue, where this cell normally occurs. He has pointed out that frequently in the spleen or other organ a definite mass of these cells is formed; this mass he designates 'clasmatocytic tissue'. This tissue reaction is sometimes disproportionate to the number of parasites present, and the cells in the centre of an island of macrophages are quite frequently entirely free from parasites. Meleney considers that this is a definite tissue response, as the formation of these masses of macrophages appears to precede invasion of the parasites. No mitotic figures have been observed in these masses, but it seems difficult to explain this accumulation unless there is local multiplication.

The principal changes that occur in the more important organs are given below:

SPLEEN

It is almost always enlarged; it may be immense, weighing as much as ten pounds in the case of an adult.

The capsule is usually thickened, and occasionally at the site of recent perisplenitis there is marked thickening. The consistency is a variable quality, but in the majority of cases it is soft and pulpy, the spleen surface bulging on section of the capsule. In other

instances it is firm, retaining its shape on removal from the body, but it is usually very friable. And again in some instances it is hard and fibrous. The cut surface has a uniform dark red appearance; if the knife is drawn across the cut surface of the soft type of spleen, quantities of pulp will be scraped off and the surface will be felt to be quite smooth, whereas in the case of the hard spleen, no pulp will be scraped off and the surface will feel gritty. There may be infarcts.

Microscopically, there is marked increase in the fibrous tissue. There is infiltration by masses of heavily parasitized macrophages; these encroach on the Malpighian corpuscles, which are eventually completely replaced; in many cases these are scarcely visible. There is considerable congestion of the vascular spaces. The parasitized macrophages appear to dominate the whole picture.

LIVER

The organ is usually enlarged. It is firm, retaining its shape well on removal from the body. It is friable, but not as friable as the spleen. The capsule is thickened in places, and when cut across the liver displays the greasy appearance associated with fatty degeneration. It also shows the nutmeg appearance of the chronically congested liver.

There is usually fat infiltration of the liver cells, which may be invaded by leishmania in the later stages of the infection. Kupffer's cells in the blood vessels are heavily parasitized. The endothelial cells of the vessels are swollen, proliferated and invaded by leishmania; occasionally the lumen of the vessels appears to be blocked by proliferated endothelial cells. In a few advanced cases the parenchymatous cells are replaced almost entirely by fibrous tissue and parasitized macrophages.

BONE MARROW

There is usually increased activity of the bone marrow, which is red and soft, and a decrease of the fat. There are large numbers of erythroblasts, myelocytes and macrophages; the whole marrow may be invaded by tightly packed macrophages. There are frequently large numbers of parasities; these are mostly in the macrophages, but occasionally they may be observed in the myelocytes and polymorphonuclear leucocytes.

LYMPHATIC GLANDS

There is no constant microscopic change in the lymphatic glands; the mesenteric glands are frequently observed to be enlarged with necrotic areas in the centre. There is usually proliferation of the macrophages between the follicles, which compress and eventually invade the lymphatic tissue and tend to disorganise the normal structural arrangement of the lymph follicles. The parasites are usually scanty and confined to the macrophages.

INTESTINAL TRACT

Christophers (1904) reported that ulceration of the large intestine was a constant feature of post-mortem examinations of persons who had died of kala-azar; the ulcers were usually deep and sloughing. The present writer has seldom observed this condition. Occasionally, small superficial ulcers have been seen in both the large and small intestines, but whenever extensive ulceration has been present it has been in cases in which there has been clinical dysentery during life, not a particularly common complication in hospital-treated cases.

Marrion-Perry (1922) cut sections of the jejunum of a patient who had died of kala-azar in India, and showed that the villi were distended with proliferated and parasitized endothelial cells; his sections showed that there was no epithelium covering the villi, so that the parasitized cells were in direct contact with the intestinal contents. Meleney (1925) is of the opinion that the absence of the epithelium was due to post-mortem changes or was rubbed off during the manipulation of the tissue, as the epithelium is intact at the bases of the villi. In the hamster he found similar changes, but by rapid fixation he was able to preserve the epithelium intact. Invasion of the submucosa by parasitized macrophages occurs in all parts of the intestinal tract, but is most marked in the neighbourhood of Peyer's patches and solitary follicles. Occasionally, the lymph follicles are invaded by parasitized macrophages.

SUPRA-RENALS

The changes that occur in these glands are not constant, but have been observed in the majority of the cases examined. There is decided thickening of the capsule. There are no very marked

changes in the *zona glomerulosa* of the cortex, but in the *zona fasiculata* there is marked separation of the columns, with an increase of soft fibrous tissue and an invasion of the interstitial tissue by macrophages. The same condition is noticeable in the medulla, but here it is less marked. The parenchymatous cells are occasionally invaded by leishmania, but the parasites are usually observed only in the macrophages.

THYROID

There is an increase in the intralobular stroma, so that the acini are cut off into irregular masses. Some of the acini form retention cysts; in some instances these are infiltrated with leucocytes, but in others there is no infiltration. Other acini contain no colloid. Occasionally, there is infiltration of the acini by macrophages, and in some areas the parenchyma is entirely replaced by proliferating masses of these cells.

KIDNEYS

There is not usually any marked microscopic change here. There is an invasion of the interstitial tissue by parasitized macrophages, and there is sometimes intratubular œdema. The glomeruli seldom show any change, but there is often cloudy swelling of the secreting tubules.

HEART

The heart is usually dilated and flabby, and in some instances the appearance suggests brown atrophy. There is infiltration of the myocardium and subendothelial tissue by parasitized macrophages. In some instances there is a certain degree of myocardial degeneration—the cross striations of the muscle fibres being indistinct. Parasities have not been observed in the endocardium.

TESTES

Shortt (1923*c*) reported the finding of parasites in the interstitial cells of Leydig, but Meleney (1925) is of the opinion that the parasites are confined to the invading macrophages, and considers that Shortt failed to distinguish between these two groups of cells.

There are no characteristic changes in any other organs or

tissues of the body, but parasites have been found in the interstitial tissue in the pancreas, lungs, prostate, urinary bladder, and in the capillaries of the meninges and choroid plexus.

POST-KALA-AZAR DERMAL LEISHMANIASIS

(*a*) *The Early Depigmented Stage.*—The epithelium has undergone very little change, but there is less pigment in the cells of the basal layer in the leucodermic areas than in those of the normal skin of the same individual. There is œdema of the sub-papillary tissues, and the vessels of this layer are large and dilated. Below this there is a certain amount of infiltration of macrophages in the region of the sub-papillary plexus; the white and elastic fibres have been destroyed in this area, and it is probable that the function of the melanoblasts has been interfered with.

No parasites have been observed in sections of the tissues in this stage, but cultures of leishmania have been obtained.

(*b*) *The Nodular Stage.*—The epithelium is flattened and thin; it consists of a basal layer containing very little pigment, a couple of layers of prickle cells and a thin covering of horny cells. The sub-papillary layer is œdematous and the fibrous and elastic tissue has atrophied. Melanoblasts are well seen. Below this œdematous area is a granulomatous mass consisting largely of proliferating macrophages and fibroblasts. In the centre of this mass, here and there, are multinucleate cells packed with parasites; away from the centre of the nodule the macrophages contain fewer parasites and the cells at the periphery do not appear to be parasitized.

In the case reported by Shortt he noted that the parasites were more abundant in the superficial part of the nodule immediately under the epidermis.

(*c*) *The Xanthoma Type.*—Whereas in the nodular type the spread is centripetal, raising and flattening the epidermis, in the xanthoma type there is a tendency in some regions, such as the elbow and axilla, for the spread to be centifugal, forming large plaques in these areas. The histological appearances are similar to those of the nodular type, except that there is a tendency to fibrosis and constriction of the venules, with subsequent dilatation; for this reason the xanthoma plaques are a deep orange-red, whilst the nodules are a much paler orange-yellow colour.



PLATE VII

Non-ulcerative post-kala-azar dermal leishmaniasis: first stage.



PLATE VIII

Non-ulcerative post-kala-azar dermal leishmaniasis : second stage.

CHAPTER IV

SYMPTOMATOLOGY

Incubation period—Onset—The fever—Other general symptoms—Physical signs—General appearance—Spleen—Liver—Blood and circulatory system—Digestive system—Respiratory system—Nervous system—Bones—Skin and subcutaneous tissues—Urinary system—Genital system—Complications—Sequelæ—Chronic splenomegaly—Cirrhosis of the liver—Post-treatment jaundice—Post-kala-azar dermal leishmaniasis.

INCUBATION PERIOD

THERE is practically no information on this subject. Manson (1919) reports a case in which the patient became infected after 10 days' residence in an endemic area, and Muir (Napier and Muir, 1923) also refers to a patient coming from a non-endemic area who lived in an infected house, practically the only possible source of infection, for only 14 days before the first symptoms of the disease appeared. On the other hand, the writer recently had a patient under his charge who had lived for 18 months away from an endemic area before the first symptoms appeared. In another instance, to which reference has already been made, the first symptoms appeared four months after the patient had been inoculated with infected material. The generally accepted opinion is that the incubation period is usually from six weeks to four months.

ONSET

There is nothing absolutely characteristic about the onset of the disease, so much so that one can safely say that during the first month, on clinical grounds alone, it cannot be diagnosed except by sheer guessing.

It is possible to classify the modes of onset into certain types, the first two types having applied to them the name of the disease for which they are usually mistaken, that is typhoid and malaria, respectively, and the third being classified under the general term 'insidious'.

Typhoid Type.—In an endemic area this type of onset will

account for about 20 per cent. of the cases. The patient becomes ill with a rapidly climbing fever, which reaches 103°, 104°F., or higher, after a week; a high continuous or high remittent fever continues for ten days or so, and then slowly falls to the 99-100°F. line. There are seldom any abdominal symptoms, but there may be a little diarrhœa. The spleen is possibly just palpable, and there is a suggestion of tenderness, but if the patient is an old malarial subject the spleen will, of course, be larger than this. The liver is not usually enlarged. The pulse is rapid, 120 per minute, and, provided that the patient is not very constipated, the tongue is usually fairly clean. The mental condition is quite clear and the patient does not usually feel particularly ill even when the temperature is very high. The helpful points with regard to differential diagnosis at this stage are the absence of any definite abdominal symptoms, of the foul tongue and of the characteristic toxic drowsiness of a typhoid patient, and the presence of a double remittent temperature.

The temperature may now fall to normal, but as a general rule it remains about the 99° line, and after another fortnight gradually creeps up again, when a 'relapse' will be diagnosed. It is frequently not until the second 'relapse' that the true condition of affairs is suspected, as the spleen, which has been steadily growing, is now very distinctly enlarged.

Malarial Type.—This is the commonest type of onset in an endemic area. As districts that are endemic for kala-azar are nearly always endemic for malaria, though the reverse is of course not the case, it is quite easy to understand the popular belief, which is still also held by a few practitioners, that kala-azar is only a chronic or 'neglected' form of malaria. This form of onset is like an ordinary tertian or quartan malarial attack, the fever rising suddenly to 102° or 103° accompanied by a rigor, but not usually followed by sweating, and dropping on the following day, or the same day, to normal, then possibly rising on the third or fourth day, and after that becoming somewhat irregular. Quinine will have a temporary effect; in fact, it may almost appear to cure the first attack altogether, but when another attack comes on, about a week later, quinine will have little or no effect on it. This type is seldom diagnosed on clinical grounds alone until the quinine-resistant nature of the fever is definitely established, though a household history of kala-azar may give rise to suspicion.



Plate V

Insidious Type.—This last type of onset is so ill-defined that even from the most careful inquiry it is often impossible to date the real beginning of the disease. The patient may have had a feeling of ill-being for some months, without any definite symptom which would cause him to seek medical advice, even were he in a position to obtain it. He may have an occasional attack of fever, which disappears with or without a dose of quinine. He will then suddenly get an attack of one of the commoner complications, such as dysentery or pneumonia, and this will cause him to seek medical advice. The nature of the condition may not at first be suspected, but when the more acute symptoms of the complication clear up and the condition of the patient does not improve very much, or improves only temporarily, then suspicion may arise. The spleen, and the liver as well, will probably be enlarged, and, as the disease is in a comparatively advanced stage by now, other physical signs and symptoms will also be present.

Before leaving the question of the onset of the disease there are one or two points that might be mentioned. There are some cases in which the disease has, strictly speaking, no clinical onset. The patients have no symptoms of the disease, but on physical examination they are found to have an enlarged spleen. Spleen puncture reveals the presence of the infection. The writer has seen a few cases of this nature, and Knowles (1920) reports one. It is improbable that there are many such cases, but, until a number of spleen punctures or blood cultures have been carried out on apparently healthy persons in an endemic district, one cannot safely say that this condition is rare. The rapid type of onset is more frequently observed under epidemic conditions, that is to say when the disease is established on virgin soil. In an endemic area children and immigrants, more especially the latter, are most prone to this form of attack. In Calcutta it has been noticed that when Chinese are infected the disease takes on a rapid form, which with corresponding rapidity responds to treatment.

THE FEVER

An attempt to classify the types of fever that are observed when once the disease is well established merely resolves itself into a matter of making the maximum number of variations, by combin-

ing the words 'high' and 'low' with the words 'continuous', 'remittent' and 'intermittent', and interposing the words 'double' and 'triple' wherever suitable. There is, however, one form of fever which is characteristic of the disease, that is, the double intermittent or remittent type of fever. The temperature subsides towards early morning, and remains low until about midday. It rises in the afternoon, subsiding again towards evening. About eight or nine at night it again rises, or the second rise may be delayed until midnight, and the temperature again subsides towards morning. In order to demonstrate this double rise it may be

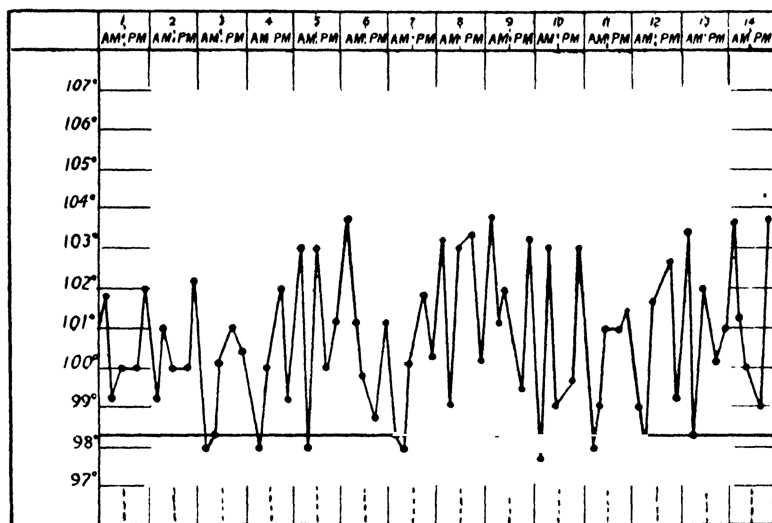


CHART VII

Four-hourly temperature chart showing characteristic double rise in 24 hours.

necessary to take the temperature every three hours, day and night. Chart VII is copied from the four-hourly temperature record in a case in which the double daily rise is well demonstrated.

Some writers have exaggerated the diagnostic value of this sign, the double diurnal rise of temperature. It is possibly a sign which is present at some time or another during the course of the disease in the majority of cases, and it is a sign of great diagnostic value when it is quite definitely present, but its absence is not a matter of

great importance, as the chances of the condition being present at the time the patient is under observation are comparatively small. In the Carmichael Hospital for Tropical Diseases, where a four-hourly temperature chart is kept, a definite double diurnal rise is observed in less than 20 per cent. of the kala-azar cases during their stay in hospital.

There is throughout the course of the untreated disease a tendency for big waves to appear on the temperature chart. There

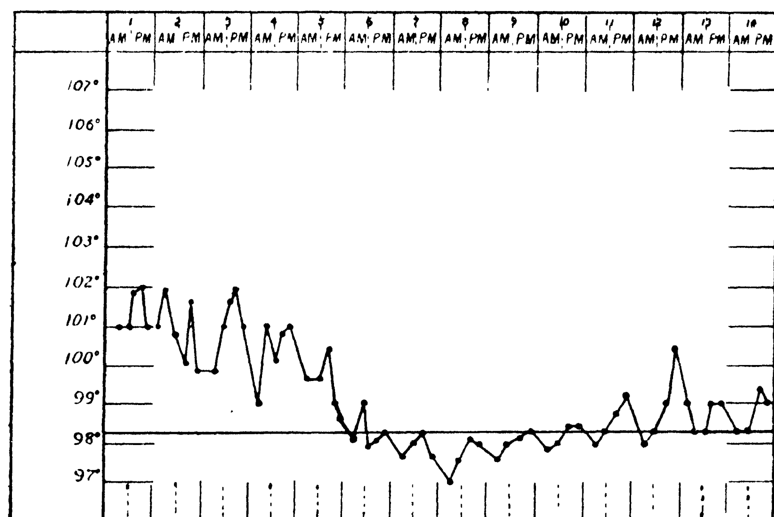


CHART VIII

Four-hourly temperature chart showing an occasional double rise and an afebrile period.

is seldom any regularity or periodicity in these waves, but, at the commencement of the disease at least, they repeatedly occur. During the last year patients have been kept under observation in the Carmichael Hospital for long periods—up to two months—and more than half of them, patients who have been admitted with a high temperature, have, without any specific treatment, become apyrexial for periods of some days.

During the apyrexial period the characteristic nightly rise of temperature may persist. During such intervals, unless taken at

midnight, no rise of temperature may be recorded. The patient and his friends may be quite unaware that there is any fever, as they are asleep at the time. The second rise at night is most characteristic of kala-azar, and we are not aware of any other disease where it is present. The nightly rise is also the last to persist when the patient is under antimony treatment and is recovering. He will often then complain of profuse sweating in the early morning, even when no rise has been recorded on the thermometer.

Sometimes a third diurnal rise of temperature may be recorded, and this, though equally diagnostic, is not as frequently seen as the double rise.

There are some cases which are definitely apyrexial, at least from the time that they come under observation in hospital, even a two-hourly temperature chart failing to demonstrate any rise above normal for some weeks at a time.

There is one very characteristic point about the fever, which has already been mentioned but is worth emphasising; it is that the patient with a temperature of 102° may be doing his work in all the ordinary way and be quite unaware that he has fever. We have seen a child playing cheerfully in the ward with a beaming smile on his face and a temperature of 104° F. The association of headache with this fever is very much less common than in the case of a fever due to any other cause.

The Effect of Quinine.—At this stage the fever is usually absolutely quinine-resistant. In the days before antimony was introduced for the treatment of the disease, heroic dosage with quinine was reputed to check the fever and even in some instances to cure the disease, but these isolated instances do not in any way impair the general truth of the above statement.

OTHER GENERAL SYMPTOMS

A patient in whom the disease has reached a comparatively advanced stage will complain of progressive loss of weight, increasing darkness of the skin—usually noticed by his friends—falling of the hair, palpitation of the heart and shortness of breath, increased appetite but a poor digestion—a condition which naturally leads to intermittent attacks of diarrhœa—bleeding from the nose



Plate VI

and from the gums, and a persistent very irritating cough. Headaches, which one would expect to get with the fever, are noticeably absent in most cases. The patient will also usually complain of progressive enlargement of the spleen, in some cases this being the first symptom noticed and in others its presence not having been noted for a month or two after the commencement of the fever.

PHYSICAL SIGNS

(It must be understood that, unless otherwise stated, the condi tion described is that of a patient in whom the disease is well advanced, the condition in which you may expect an average patient to be in whom the disease has been allowed to advance unchecked for about six months. It is never possible to say exactly what stage the patient will have reached in any given time, as in some cases it advances very rapidly and in others very slowly.)

General Appearance.—The patient is weak and emaciated; the hair is dry, lustreless and scarce; the natural pigmentation in dark-skinned people of the skin of the forehead, the temples and round the mouth is intensified, and this pigmentation is shown up by contrast with the bloodlessness of the less pigmented part of the face, like the appearance given by shading a white paper lightly with a black lead pencil, and in some children the presence of adventitious hair is well marked; there is marked visible pulsation of the carotids in the neck and the rapid pulsation of the heart is observed through the thin chest wall; the abdomen is protuberant, with the enlarged spleen and liver outlined on it; the cutaneous veins on the lower part of the chest and upper part of the abdomen stand out; the legs are miserably thin, with tight shiny skin stretched over the shins; and the feet are possibly œdematous.

Spleen.—The spleen is enlarged and palpable in the majority of cases of the disease, the exceptions are those cases in which the liver appears to be bearing the brunt of the disease and is very markedly enlarged, in those in which, due to some unusual thickening and adhesion of the peritoneum, the spleen is prevented from enlarging downwards but enlarges upwards, and finally in that unusual type of the disease in which there is no organic enlargement at all.

Size.—As a rule the spleen enlarges with the regularity and precision of a gravid uterus, reaching the level of the costal arch at the end of the first month, being palpable one inch at the end of the second, two inches at the end of the third, and so on. There are, however, exceptions to this general rule. There is practically no condition in which so rapid an enlargement of the spleen can take place, from being just palpable a spleen will sometimes reach the level of the umbilicus in a month, and, on the other hand, there are cases in which the enlargement is slow or is checked by the intervention of some inflammatory complication, such as broncho-pneumonia or cancrum oris. The actual size of the spleen will not be found to be a very useful diagnostic point, although the regularity and comparative rapidity of the enlargement may arouse suspicion. The teaching which was common in Medical Schools in India, that a spleen above the umbilicus is probably malaria and a spleen below that level is kala-azar, will certainly not hold in these days, when the disease is recognised in a much earlier stage than it used to be. In the analysis of 417 suspected cases (Napier, 1922) in which a spleen puncture was done, it was found that in 286 kala-azar cases the average size of the spleen was 5·47 inches below the costal margin, whereas in 131 non-leishmania cases, mostly malaria, it was 5·95 inches; and that in the former group, 9·7 per cent. had spleens of eight inches or more, below the costal arch, whereas in the latter group 18·3 per cent. had spleens of this size. As the diminution in the size of the spleen is a very useful indication of the progress of the patient under treatment, it is necessary to adopt some standard method of measuring the size of the spleen. The method that we adopt is as follows:

In the first place, the patient must be horizontal, otherwise the position of the spleen may be grossly altered. A line is drawn from the umbilicus towards the left side of the costal arch, dividing equally the angle between the vertical and horizontal lines through this point; the point at which this line cuts the costal arch, which will be about the tip of the ninth rib, should be taken as the fixed point, and from this the distance to the lowest point of the spleen is measured. This, of course, does not allow for the upward enlargment of the organ, but it is a very rapid method, much more rapidly done than explained, and one which, with very few exceptions, gives a very accurate idea of the proportionate size of the

spleen. If there is any downward dislocation of the spleen, the organ should be held in place while the measurement is being made. This precaution will be found very necessary as the spleen diminishes under treatment, especially in the cases where there has been any considerable enlargement. In these cases the splenic ligaments have become stretched, and naturally do not contract as rapidly as the organ itself diminishes in size.

Frequently at the end of a course of treatment the spleen may still appear to reach to the umbilicus, but if one can get one's fingers under the lower edge it will be found that the organ is very flat, and can, without the exercise of any pressure, be replaced under the costal arch.

Consistency.—The peculiar soft, doughy feeling of a kala-azar spleen will be found an extremely useful diagnostic point; it is not common in other conditions, whereas the wood-like resistance of an old-standing chronic malarial spleen is uncommon in even a fairly chronic case of kala-azar. 'The more chronic the disease the harder the spleen', may be taken as a general rule.

Tenderness.—This is not common, and is not complained of in more than about five per cent. of cases. It is of no value as a diagnostic point, as perisplenitis occurs as often in other similar diseases. Occasionally, however, a patient, either under treatment or during the course of the disease prior to the commencement of treatment, complains of a pain in his spleen, which comes on suddenly and may last for a few days. The pain is at first general, but soon becomes localised to one particular spot. This pain is probably caused by the sudden blocking of one of the splenic arterioles by the invasion of its endothelium by the parasites of the disease, with the consequent formation of an infarct.

Liver.—There is nearly always some enlargement of the liver in this disease. In 300 cases seen in Calcutta the liver was palpable in 88 per cent., measurably enlarged in 64 per cent., and enlarged more than three inches in 20 per cent. of cases. An enlarged, thinned out, soft liver overlapping a large soft spleen is a condition which is very characteristic of the disease. A certain degree of tenderness is sometimes present, but this is not in any way comparable with that which would be found in liver abscess.

The sharp lower edge of the liver will help to distinguish this condition from the passive congestion that is met with in malaria.

A measurable degree of enlargement of the liver is not common in uncomplicated malaria, but, nevertheless, as a practical diagnostic point we have found it a somewhat disappointing sign. In the series of 140 patients not suffering from kala-azar, for the most part cases of chronic malaria, who attended the kala-azar clinic at the Calcutta School of Tropical Medicine, 86 per cent. had palpable livers. Occasionally liver enlargement takes the place of splenic enlargement, but as a general rule both conditions are present.

Blood and Circulatory System.—(The cytological and bio-chemical changes in the blood will not be referred to in this section.) A certain degree of anæmia is almost always present. It is not, however, usually so obvious as the anæmia that is associated with other forms of 'spleen fever', and, therefore, profound anæmia is, as a rule, a point against the diagnosis of this disease.

The blood pressure is usually low, the systolic pressure being below 100 mm. of mercury. Hæmic murmurs of the heart are the rule, rather than the exception. The very marked pulsation of the carotids in the neck, one of the most useful clinical signs, is probably also due to this.

The heart carries a very heavy burden of toxic effects. A certain amount of dilatation is the rule, and in a few cases hypertrophy also of the heart has been noted.

Even in the earliest stages of the disease the rapidity of the pulse rate constitutes a valuable diagnostic sign.

Œdema of the extremities is comparatively common; it was found in 16 per cent. of 300 Calcutta cases at the time of examination, but a very much greater number gave a history of having had swelling of the feet at one time or another.

Clinically obvious ascites is not common, being found in less than three per cent. of the Calcutta cases.

Bleeding from the gums and epistaxis are the general rule. The appearance of purpuric spots is not very uncommon. This last named condition was, in the days prior to the introduction of the antimony tartrate treatment, very often a terminal symptom of the disease, and even in these days we have seen cases, which have only come under our treatment when the disease was almost in its terminal stage, suddenly develop purpuric spots, uncontrollable hæmorrhage from the gums and hæmorrhage into the bowel, the

condition being almost that of Henoch's purpura. Purpuric spots also appear occasionally in cases which are progressing satisfactorily under treatment, usually on the legs, especially in cases that have at one time suffered from œdema, on the chest or abdomen and on the arms and hands, in the latter case after a knock of a degree that would otherwise not even cause a bruise. The appearance of these spots has been noted to coincide with sudden diminution in the size of the spleen, and certain other conditions which suggest the possibility of protein poisoning due to rapid plasmolysis having occurred. Further details will be given of this condition under the heading of treatment.

Marked congestion of the abdominal veins has already been mentioned. This is most common in children.

Digestive System and Alimentary Tract.—Gingivitis, with subsequent loosening of the teeth, is common. Stomatitis, other than cancrum oris, is not very uncommon at any stage, and in the late stages cancrum oris is the most classical and most fatal complication of the disease. The last named condition is not, however, seen as often as it used to be in the days before a satisfactory form of treatment was introduced. As in the case of other complications of this disease, it is due to the low resistance of the tissues, which in its turn is probably due to the leucopenia and especially to the shortage of polymorphonuclear leucocytes. Cleanliness of the tongue is one of the most striking features of kala-azar as distinguishing it from pyrexia due to other causes.

The appetite is usually very good, again an unusual condition in company with pyrexia, but the digestion is very frequently bad. The patient is always hungry, but when the most tempting food is set before him he only takes a very little of it.

Diarrhœa and dysentery are so common that they are looked upon by some observers as an essential part of the disease, but the specific nature of these complications has never been proved, and it is usually possible to find active *Entamœbæ histolytica* or to isolate one of the commoner dysentery bacilli from the mucus passed in the stool of a patient suffering from a severe attack of dysentery.

Diarrhœa is frequently the symptom that first causes a patient to seek medical advice and, on the other hand, it is very commonly a terminal complication.

Respiratory System.—The respiratory system is peculiarly

prone to inflammatory processes in this disease. In the early stages, and at all stages of the disease, an irritating cough is usually present without any marked physical signs in the lungs to account for it. In a few cases this is the most distressing symptom of the disease, seriously interfering with the patient's rest at night. It is probably due to irritation of the vagus from pressure caused by the enlarged spleen.

In the later stages a certain amount of congestion of the bases of the lungs is common, and bronchitis is quite frequent. Pleurisy, with or without effusion, is occasionally seen, but these two last conditions should be rather classed as complications.

In the final stages dyspnœa is frequently noticed, due in part to the anæmia and probably also to the œdema of the lung which is liable to occur at this stage. As a terminal complication pneumonia is very common, and will be referred to again under this heading.

Nervous System.—This seems peculiarly free from attack by the parasites or their toxins. The mental condition is always quite clear even in the final stages of the disease, and delirium is less common during pyrexial attacks in this disease than in any other. This fact forms a point of diagnostic value.

Herpes zoster occurs sometimes during the course of the disease in a patient who is not undergoing treatment, but it is a condition which is much more frequently observed in a patient receiving antimony injections; the condition usually clears up on the antimony being discontinued.

Retinal hæmorrhages have been observed in a few cases.

Bones.—The red bone marrow as a blood-forming organ is attacked in kala-azar. Pains in the bones occur, but their occurrence is a point of little diagnostic value. An unusual tenderness of the long bones, especially the tibiæ, is very frequent, and is almost an invariable condition in children. A very distinct pitting over the tibiæ is usually felt if firm pressure is caused with the thumb. It seems probable that this is due to œdema of the periosteum of the tibiæ, caused possibly by changes in the red bone marrow. Necrosis of the lower jaw has occurred, but this was due to extension of the septic condition from the mouth.

Skin and Subcutaneous Tissues.—Certain very marked changes, probably of a trophic nature, take place in the skin of a kala-azar patient.

(*a*) The whole skin surface becomes dry, rough and harsh. The hair falls out and becomes very thin indeed; we have seen children become almost bald. Skin eruptions are common, and all sores that form are slow to heal. The parasites can sometimes be found in the granulation tissue, but their presence here is probably not due to any special local deposition, but to the presence of the parasites in the general circulation. Acarus infections and septic folliculitis are very common, but these are probably due rather to the habits of the patient than to any special liability of the tissues to attack by these organisms. It is the habit amongst some people in this country never to allow a patient who is suffering from fever to have a bath. This may be a satisfactory rule in the case of malarial attacks, where the fever comes suddenly and as quickly disappears, but it is a very disastrous principle when applied to a kala-azar patient, who may suffer from continuous fever for a number of months. Children have been admitted to the Carmichael Hospital for Tropical Diseases applied to whom the word 'black fever' was most appropriate: their skins, naturally comparatively fair, had become, with the exception of the palms of their hands and the soles of their feet, a dull, earthy black. This blackness proved not to be even skin deep, although to remove it completely, a process which sometimes took weeks, the superficial layer of the skin had to be sacrificed.

(*b*) The characteristic blackening of the skin from which the disease derives its name is possibly due to a certain extent to increased activity of the melanoblasts, but is also an intensification of the natural pigmentation due to the dryness of the skin. It is most marked over the forehead and temples, and occasionally around the mouth. The blackening is intensified by contrast with the anæmic pallor of the rest of the face. This pseudo-pigmentation is not seen in pure-blooded Europeans, but is very marked in dark skinned Anglo-Indians.

The skin over the tibiæ is stretched and glossy. This condition is usually associated with pitting of the periosteum of the tibiæ, referred to above. Jaundice is not uncommon, though often a temporary symptom.

Urinary System.—There are a number of symptoms occurring during the course of the disease which suggest renal inefficiency; puffiness of the face, swelling of the legs, and a certain

degree of ascites are all common, and are sometimes associated with a decreased output of urine. It is probable that the œdema is due to vaso-motor disturbances, as the urine seldom provides evidence of any serious renal dysfunction.

Genital System.—Amenorrhœa is often an early symptom, and is an almost invariable one in a well-established case.

Whilst it seems probable that conception is prevented in the later stages of the disease when amenorrhœa is established, we have seen a number of cases in which conception occurred early in the disease, and in which an uncomplicated pregnancy was continued to full term and was ended by the birth of a comparatively healthy child.

An instance in which the disease was apparently transmitted *in utero* has recently come under the writer's notice. A European female patient, who had been seen by the writer in consultation in Calcutta and had been given a short course of treatment for kala-azar, proceeded to England, where she was confined; subsequent to her confinement the disease relapsed, but she was eventually completely cured. Both the mother and child remained in England, and when the latter was nearly a year old it was found to be suffering from kala-azar; the diagnosis was confirmed by spleen puncture.[1]

On the other hand, Muir (Napier and Muir, 1923) reports the case of a pregnant woman who died of kala-azar; a post-mortem was performed, and no trace of leishmania was found in the fœtus.

IMPORTANT CLINICAL DIAGNOSTIC POINTS

The marked emaciation, the scanty hair, the dark skin, the pulsating carotids in the neck, the rapid pulse, the enlargement of the liver, the peculiar soft, doughy feeling of the enlarged spleen and the double remittent and quinine-resistant nature of the fever are the most useful diagnostic points, but there is absolutely no sign nor symptom of the disease which may not be absent.

COMPLICATIONS

The resistance of the tissues to bacterial invasion is very much lowered in kala-azar, and practically all the complications that occur can be attributed to this fact. Virulent infections, as, for example, epidemic influenza, may prove very fatal in a kala-azar hospital, and

[1] Details of this case have recently been published (Low & Cooke, 1926).

even milder infections are liable to gain a sound footing and to be difficult to dislodge.

Respiratory Tract.—About 90 per cent. of kala-azar patients have some degree of bronchial irritability, but with few physical signs. Broncho-pneumonia and lobar pneumonia are common complications, both in treated and untreated cases. The latter condition very frequently proves fatal, but, should the patient recover, his condition will have been considerably benefited; the benefit, however, is seldom permanent, and a full course of treatment should not be withheld on account of the temporary improvement that has been noted.

Œdema of the glottis is a comparatively rare complication, but in three cases in which the writer saw it it proved rapidly fatal. It was particularly distressing, as these cases were otherwise progressing very satisfactorily.

Alimentary Tract.—Cancrum oris occurs in a small percentage of cases in which the condition has been neglected for a considerable time. It is often, but by no means always, a fatal condition.

Rogers (1910) places the incidence of cancrum oris at 17 per cent., but in these more enlightened days the incidence is very much lower. It was noted in less than 2 per cent. of the Calcutta series of 300 cases.

Indigestion is a common complaint of the kala-azar patient.

Dysentery makes its appearance at some stage of the disease with such unfailing regularity that it has very frequently been suggested that it is an essential part of the disease. A few cases give a history of the disease having started with an attack of this nature, it is quite often an incident during the course of the disease, and is very frequently a terminal condition.

Experience has shown that a secondary causative organism can usually be found to account for these dysenteric attacks.

During the course of the disease a watery diarrhœa is not at all uncommon.

Hæmorrhages.—Hæmorrhages may occur from any part of the body. Bleeding from the gums and from the nose is common. Large purpuric patches appear anywhere on the skin surface, but usually at a spot which has been subjected to slight injury. A very common place is around the ankles, when this has recently been the site of œdema. Melæna occurs occcasionally, and we

have seen cases which have ended fatally with a condition that simulated Henoch's purpura.

Other Inflammatory Conditions.—Otitis media is a common complication, and occasionally a mastoid abscess will form in cases suffering from this condition.

Œdema and Ascites.—Œdema of the feet is a very common complication, and general anasarca is occasionally present. The cause of these conditions is not clear. The presence of albumen in the urine is not noticeably associated with them; nor are there usually signs of heart failure. Ankylostomiasis will account for a large percentage of those cases. A transitory œdema of the feet appearing during the course of treatment is common. A few cases of acute nephritis have been described, but they were probably an accidental association. The writer has had no experience of this complication.

A definite progressive ascites, unaccompanied by general anasarca, is seen occasionally in advanced cases. It is probably due to cirrhotic changes in the liver. The prognostic significance of tnis complication is very serious, but the condition should not be confused with the lesser degree of ascites due to a subacute inflammatory condition of the peritoneum or with that accompanied by general anasarca.

SEQUELÆ

The satisfactory form of treatment for kala-azar has not been in practice for a sufficiently long time for us to have a thorough knowledge of the eventual fate of cured cases of the disease, but, from our experience up to the present, there is every reason to suppose that the large majority of cases recover completely and return to their original state of health. In the more chronic form of the disease, in which the patients have developed a hard fibrous spleen, this organ probably does not return to its normal size. There are a few conditions which can be looked upon as sequelæ of the disease, namely:

Chronic Splenomegaly plus Severe Anæmia.—The writer has seen a few cases which have been at one time authentic cases of kala-azar, and have been treated and cured of this condition, who now have huge spleens and show signs of anæmia of the pernicious type. The patients had no fever, but were clinically

otherwise very like kala-azar. The white blood count was about 2,000 per c.mm. Repeated spleen punctures, as many as four in one case, failed to demonstrate the presence of the parasites, and the aldehyde tests were negative in every case. All forms of treatment had little effect, and the patients were discharged from hospital in much the same condition as they were admitted. One patient, originally treated by Sir Leonard Rogers in 1918, has been under the writer's observation for the last five years; his condition has remained practically unchanged throughout this period.

Cirrhosis of the Liver.—A certain number of patients are seen who give a history of fever on and off for a few years, have marked ascites, enlargement of the spleen and low irregular fever; the aldehyde reaction is usually positive and there is leucopenia. A spleen puncture smear will often fail to show any parasites, but a culture on N.N.N. medium will usually show a flagellate growth. In a few cases no leishmania infection can be demonstrated. These patients do not usually improve under antimony treatment, but if no specific treatment is given they linger on for many months, and in some cases probably years.

When these cases come to the post-mortem table it is found that there are extensive fibrotic changes in the liver, and that the liver cells have been to a large extent replaced by fibrous tissue.

Clinically there is so little difference between the cases that do, and those that do not, show leishmania that it seems probable that the latter are cases in which the parasite has died out.

Post-treatment Jaundice.—A large percentage of patients who have undergone treatment for kala-azar have a typical attack of catarrhal jaundice within three months of the conclusion of the course of injections. The first symptom is fever, which may last a week or so; this is accompanied by pain in the liver region. As the fever subsides the patient becomes jaundiced. There is sometimes slight enlargement of the liver, but in most cases the gall-bladder is distinctly palpable and tender. The other symptoms disappear quite rapidly, but the jaundice often persists for some months.

Post-Kala-azar Dermal Leishmaniasis.—Attention was first drawn to this condition as a sequel to kala-azar by Brahmachari (1922). He described a case in which the patient had been treated for kala-azar in 1918, with potassium antimony tartrate.

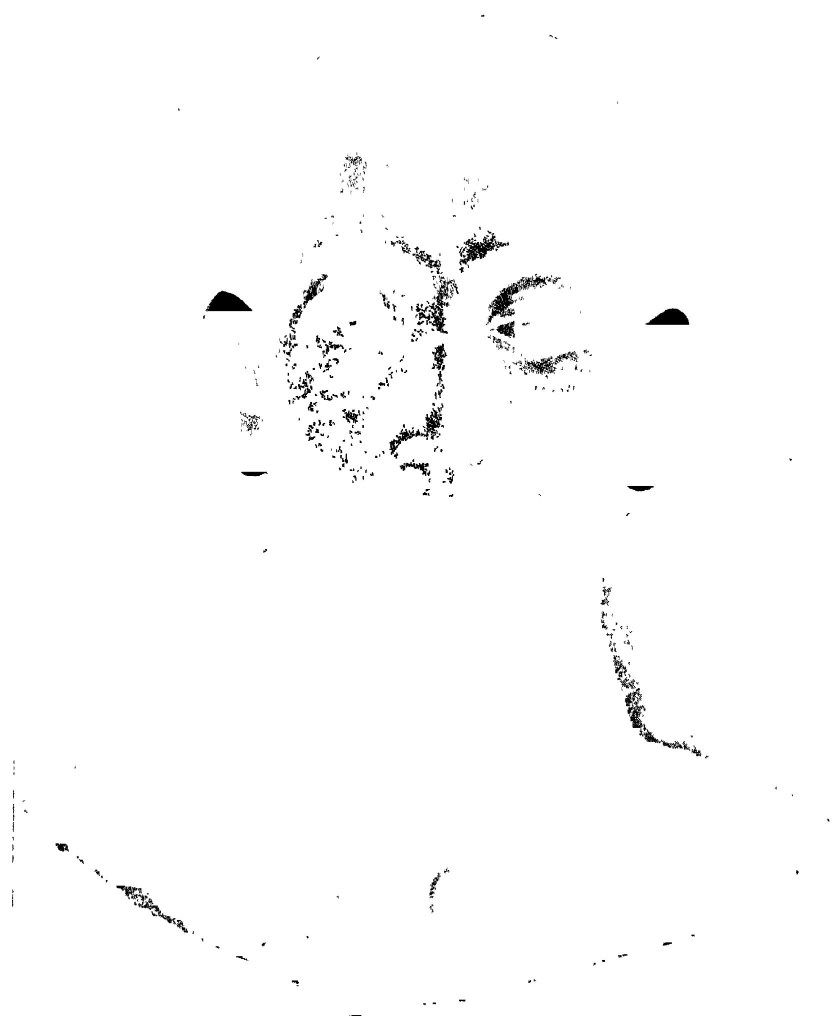
White patches appeared on the patient's face about a year after the treatment had been completed; this condition spread to other parts of the body, and subsequently papillomatous nodules developed all over the face, trunk and limbs. There was no ulceration and no anæsthesia or hyperæsthesia. The patient was at first treated for leprosy. Later smears were made from the nodules; no lepra bacilli were found, but large numbers of leishmania bodies were seen.

Since this case was shown we have seen a large number of such cases at the Calcutta School of Tropical Medicine. Various degrees of the condition, which probably represent various stages in its development, have been seen; in one case the condition resembled xanthoma tuberosum multiplex, in a number of cases leprosy, and in others, in which the condition had not progressed past the depigmented stage, leucoderma. The condition is usually so characteristic and the history so uniform that a diagnosis can be made with certainty prior to the microscopic examination of the nodules. The patients give a history of the first appearance of the depigmented patches about one year after treatment has been completed and all symptoms of kala-azar have disappeared. The nodules appear in the place of the depigmented patches, and more depigmented patches appear on other parts of the body. Some patients have observed that the nodules appear in crops. The patient with the xanthoma-like condition said that he had been suffering for about thirteen years; but nodules usually appear first about two years after the conclusion of the kala-azar treatment.

At no stage is there any tendency towards ulceration.

The condition reacts slowly to treatment; the nodules disappear first, but a certain amount of depigmentation usually remains. In the case referred to above, in which the condition resembled xanthoma tuberosum multiplex, a considerable amount of staining of the skin remained.

Although the majority of the patients give a history of having had an attack of kala-azar and of having undergone a course of treatment, there are a few instances in which the patient gives no history of having had the disease and has certainly not received treatment for it. All the cases that have been seen have been in persons who have been living in a kala-azar endemic area and have quite possibly suffered from an abortive attack of the disease.



The organisms are usually present in fair numbers in the well developed nodules, and a growth of flagellates can be obtained on N.N.N. medium, but no growth has been obtained from either the spleen puncture material or from the blood in these cases; it is probable that the parasites are confined entirely to the skin.

In the findings in these cases Brahmachari seems to see the proof of the identity of the parasites *Leishmania donovani* and *Leishmania tropica.* The writer does not interpret the findings in this way. On the contrary, it seems to prove that even when the parasite *Leishmania donovani* is localised to the skin it is not capable of producing the lesion which is always associated with *Leishmania tropica* infections.

CHAPTER V

DIAGNOSTIC METHODS AND LABORATORY TECHNIQUE

The Blood—Presence of parasites in the peripheral blood—Evidence of the blood film—Staining methods—Evidence of blood culture—Cultural methods—Cytological changes—Bio-chemical changes—Serum tests—Spleen puncture—Liver puncture—Bone puncture—The urine—Post-kala-azar dermal leishmaniasis.

THE BLOOD

PRESENCE OF THE PARASITES IN THE PERIPHERAL BLOOD

THE presence of the parasite in the peripheral blood in kala-azar has been a matter of discussion and of difference of opinion between various observers for a considerable time. Now, however, most of these differences of opinion have been brought into line.

The Evidence of the Blood Film.—Working in Madras, Patton (1914) claimed to have found parasites in nearly 100 per cent. of cases of untreated kala-azar, but in order to attain this percentage he found it necessary to examine a large number of films from each case. Mackie (1916), in Assam, found parasites in the peripheral blood in 20 per cent. of cases without a very exhaustive search. The writer's experience—mostly in Bengal—has been that, without a very thorough search, the parasites in the peripheral blood are difficult to find. These differences in the peripheral blood findings have led to the statements that the disease varies in type in these several localities. It is now clear that the differences were not in the disease, but in the observers and in other external influences. Patton, an extremely careful and conscientious observer, was stimulated in his searchings by the critics of the bed-bug theory of transmission, who suggested that the absence of the parasite from the peripheral blood in most cases was a strong argument against this theory, and further by the fact that in Madras, for some reason or other, there is a very strong feeling against performing spleen punctures; whereas the writer considers

that a spleen puncture is a comparatively safe operation, and performs it in all cases in which there is any doubt about the diagnosis.

Examinations of Blood Films.—An ordinary thin blood film, made in such a way that the majority of the leucocytes are drawn into the tail of the film, should be stained with Leishman's stain, and examined with an oil immersion lens and a low-powered eye-piece (No. 1 or 2). Patton suggested the use of a $\frac{1}{6}$th objective, but the writer has not found this satisfactory. The edge of the film only should be searched, as in this way a good deal of time is saved.

The parasites in the peripheral blood will always be found to be intracellular; occasionally, however, a large cell may have been ruptured in the act of making the smear, and in this case the parasites will naturally appear to be lying extracellularly.

Modification.—Knowles and Das Gupta (1924) have adopted a thick film method for examining for leishmania in the peripheral blood. Their technique is as follows:

(1) Four large drops of blood are placed at the corners of a small square, $\frac{1}{2}$ inch by $\frac{1}{2}$ inch, near the centre of the slide. With a round needle or glass rod they are then pooled, so that the blood covers the $\frac{1}{2}$ inch square thickly and evenly; 'puddling' must be avoided.

(2) The slides are now laid flat on the table, are covered with a Petri dish, and are allowed to dry completely. *This is the most important point of the whole process.* A thick film may appear to be dry in half an hour, but the leucocytes have not yet emigrated and become adherent to the slide. At least two hours at room temperature, or an hour in the 37°C. incubator, is required; otherwise the film gets washed away during subsequent manipulations.

(3) Lay the perfectly dry thick film, surface upwards, flat on a staining rack. Flood the slide very gently with the following mixed solution:

Glacial acetic acid,	2·5 per cent. in distilled water	.. 4 parts
Tartaric acid crystalline,	2 per cent. in distilled water	.. 1 part

This solution dehæmoglobinizes the film, and the process should be watched. An ordinary thick film will be completely dehæmoglobinized in five to ten minutes, but films with thicker patches may require a little longer.

(4) As soon as dehæmoglobinization is complete, drain off the fluid by tilting the slide. Next flood the slide with methyl alcohol. Allow it to remain on for one minute. The film is now fixed.

(5) Drain off the methyl alcohol and wash the film *very thoroughly* in distilled water. Every trace of acid must be removed from the film, or the subsequent staining will be unsatisfactory.

(6) Stain the film with Giemsa's stain, one drop to one cubic centimetre of distilled water, for ten minutes. Differentiate in distilled water. Do not blot the film, but allow it to dry in the air, placing the slide tilted against any vertical surface, with the film side downwards to protect it from dust.

(7) Examine with the $\frac{1}{12}$th inch oil immersion lens and a fairly high, e.g. No. 6, ocular. The leucocytes are seen evenly scattered over the half inch square, and can be examined rapidly for *L. donovani.* As contrasted with control thick films of healthy blood, the leucopenia of kala-azar at once becomes most strikingly apparent.

The writer has found the thin-film method more satisfactory in practice. A large number of slides can be studied in a very short time if the leucocyte edge only be examined.

The Preparation of Leishman's Stain.—The requirements: (*a*) A good quality Leishman's stain powder: this can be obtained from most English firms, but the 'Soloid' form, supplied by Burroughs Wellcome, will be found convenient and reliable.

(*b*) Pure methylic alcohol; for making a good stain this is very important.

The strength of the stain should be 0·15 per cent., that is to say, for preparing 200 c.c. of stain 0·3 gramme of dry stain will be required, or for preparing 8 ounces, about 6 grains of dry stain. The weighed amount of dry powder should be placed in a mortar, a little alcohol added, and the two well mixed by grinding the powder with a pestle. The first lot of stain should then be poured off into another vessel, a little more alcohol added, and the remaining powder ground again, and so on, until all the powder is dissolved. It is very important that the whole of the stain should be dissolved, as the solubility of the ingredients varies. The stain should be kept for at least two days before it is ready for use.

Staining Method.—It is unnecessary to fix the slides before staining with Leishman's stain. The slide is placed on a staining rack and a few drops of stain added. This is allowed to remain

for half to one minute, and then freshly distilled water is added to dilute the stain about five or six times. The staining is now allowed to continue for ten minutes, after which the slide is rapidly washed in distilled water and allowed to dry. If by any chance the stain is now found to be too light, the slide can be re-stained as above, and if it is too darkly stained it can be washed rapidly with alcohol and then re-washed with water.

The Preparation of Giemsa's Stain.

Requirements:	(*a*) Azur II eosin ..	.. 0·3 gramme
	(*b*) Azur II ..	.. 0·08 gramme
	(*c*) Pure anhydrous glycerine	.. 25 cubic centimetres

With the aid of a glass pestle and mortar the dry stain is dissolved in the glycerine; the mixture is then placed in the the incubator at 37°C. for 24 hours.

Staining Method.—The slide is placed on the staining rack and flooded with methyl alcohol; this fixes the film. The slide is then thoroughly washed in distilled water. Ten drops of Giemsa's stain are added to ten cubic centimetres of distilled water, and the slide is flooded with this dilute stain; the staining process is allowed to continue for thirty minutes, after which the slide is washed rapidly in distilled water and allowed to dry. The slide should first be examined with a ⅙th objective; if the staining is too dark the slide must be washed again in distilled water.

Evidence of the Blood Culture.—By blood culture the presence of the parasite in the peripheral blood can, according to Das Gupta (1922), be demonstrated in 100 per cent. of untreated cases of kala-azar, and also in many cases in which treatment is well advanced. The writer has found that there are a few exceptions to this rule. A few cases have been described in which the parasite was grown from the blood of patients who were apparently quite cured and had been free from symptoms for a number of months. In this latter case we must assume that the patient has become a chronic 'carrier' of the disease, but it is a somewhat confusing factor which need not be further discussed here and does not impair the practical value of blood culture as a method of diagnosis.

Method of Peripheral Blood Culture.—

Requirements.—(*a*) A small serum syringe, of capacity not less than two cubic centimetres. A Roux syringe—metal and glass—will be found to be the best. An all-glass or a Record syringe may

be used, but the Roux syringe is sterilised with less risk of breaking than either of these. The syringe, whichever is used, must first be sterilised with olive oil which has been heated to 160°C. The needle—two inches long and of medium calibre—is placed in a galley-pot containing the oil. The hot oil is then drawn up and expelled from the syringe two or three times, and the needle is attached with a pair of forceps. If the syringe is not used immediately, the needle should be passed through a flame two or three times just before being used.

(*b*) Two or three test tubes containing a solution of 2 per cent. sodium citrate and 0·85 per cent. sodium chloride.

(*c*) An incubator with a level temperature of 24°C. (This is not absolutely essential in a place in which the temperature does not fall below 18°C. or rise above 28°C. in the 24 hours.)

(*d*) A sterile Wright's pipette.

(*e*) A few tubes of N.N.N. medium.

Technique of Veni-puncture.—Venous blood is used for the culture. The best veins to choose for veni-puncture are those at the bend of the elbow, but if the patient is well covered these veins may be buried in fat, and it may be easier to puncture one of the veins on the back of the hand. The latter are very movable, and it is often very difficult to puncture the vein even when the needle is passed through the skin, as it recedes in front of the advancing needle and then suddenly slips either over it or under it.

There is a large vein at the back of the wrist, running over the outer side of the distal end of the radius between the tendons of the extensor carpi radialis longior and of the extensor pollicis brevis, which is extremely useful, especially in children, in whom it is often as big as their little finger. This vein is not usually blue, but can very easily be felt, and if congestion is caused will stand out very prominently. The one disadvantage of using this vein is that the skin over it is usually very tough.

The skin over the vein should be sterilised with iodine, which should be washed off with spirit. If the iodine is not washed off it increases the difficulty of seeing the veins. Washing the skin with xylol will increase the visibility of the veins, but is seldom necessary. Congestion is caused by putting a rubber ligature round the arm above the vein that is to be punctured. This ligature must be tight enough to stop the venous return, but not tight enough to stop



Plate X

The technique of veni-puncture.

the arterial supply to the limb. Further congestion may be caused by a gentle upward massage. The patient should either be lying down or sitting at a table with the elbow on a small pillow. The syringe should be held in the right hand, at an angle of about 15 degrees with the skin surface, and entered upwards and along the long axis of the vein. The point may enter the vein immediately or the vein may slip to one side, in the latter case the point of the needle must be made to follow the vein, pressing into the side of the vein until it is pierced.

Immediately the point of the needle is in the lumen of the vein, great care must be taken that it is not allowed to slip out, or is not pushed through the opposite wall of the vein. Children are very liable to wriggle during the operation; in dealing with them it is a good plan to hold the syringe in the right hand and with the left to grip the arm so that the back of the elbow lies in the hollow of the hand and the first two fingers and thumb can be approximated in front of the elbow. As the needle is passed into the vein the barrel of the syringe is gripped between the fingers and thumb of the left hand so that the syringe and arm cannot possibly move independently. (Plate No. X.) It will not be found necessary to grip so tightly that the venous flow is stopped. The plunger is gently withdrawn to cause negative pressure, and the blood will rush into the syringe.

Inoculation of Medium.—When two cubic centimetres, or the necessary amount, has been withdrawn it is run into sterile citrate solution, about half a cubic centimetre of blood to about ten cubic centimetres of citrate solution. This is allowed to remain in the 24°C. incubator until the corpuscles have fallen to the bottom—about two hours—or, if it is more convenient, overnight. The outside of the pipette is re-sterilised by passing it through the flame a number of times, the deposit at the bottom of the citrate solution is drawn up and inoculated into two or three tubes of N.N.N. medium, a few drops into the condensation fluid at the bottom of each tube; these are placed in the cool incubator. Needless to say, the utmost care must be taken to avoid contamination of the tubes, the cotton plugs before and after removal, and also the mouths of the test tubes before inoculation being carefully flamed.

The Examination of Cultures.—The earliest day one can expect to find any growth is the seventh, but as a general practice it is better to leave the culture untouched until the tenth day. It is not

safe to discard a tube until at least 20 days of incubation have elapsed, and examination has failed to show any flagellates. If a tube is contaminated it is usually obvious from outside inspection. Moulds growing at the top of the tube do not always affect the growth in the condensation fluid, but if there is bacterial contamination of the media, flagellates will not grow.

There will be no obvious growth on the surface of the medium, although on careful examination with a lens it is possible to see a sort of smeared appearance. The principal growth occurs on the surface of the solid part of the medium which is in contact with the condensation fluid; the latter should be examined after the surface of the medium has been scraped gently with a platinum needle. A sterilised Wright's pipette is taken, the end is broken off, and the pipette is passed through the flame before use. When cool, the pipette is introduced into the N.N.N. tube and a few drops of the condensation fluid drawn up into it. The tube is again plugged, as it may be required later. The drop of condensation fluid is transferred to a slide and a thin coverslip is placed over it. The slide is examined with a $\frac{1}{6}$th objective and a No. 4 eye-piece, or with an oil immersion objective and a lower eye-piece. The light should be cut down by lowering the condenser of the microscope. There should be little difficulty in getting the right focus, as there are always a few red blood corpuscles floating about in the fluid. It is quite easy to see whether the tube is contaminated, and if this is the case numerous bacteria will be seen, usually bacilli. If the flagellates are present they will be seen rapidly moving across the field. The uninitiated might at first mistake these for large bacilli, but if they are watched carefully it will immediately be seen that they are elliptical shaped bodies with long flagella. They will be seen to vary somewhat in size, the smaller ones being much finer, more motile and more thread-like than the larger ones, which are almost pear-shaped. The parabasal will be seen as a highly refractile point from which the flagellum appears to spring. The nucleus is not so easily seen in the unstained specimen, it lies near the centre of the parasite. The length of the parasite without the flagellum is about ten microns, that is to say about one and a half times the size of an erythrocyte. Dividing forms will often be seen, and also clumps of flagellates with the flagella centrally grouped.

Preparation of N.N.N. Medium.—There are numerous varie-

ties of medium in which the parasites can be grown, but N.N.N. is the standard medium for diagnostic purposes and is the most reliable; its preparation is, however, not very simple. This medium consists of whole rabbit's blood added to salt agar. Different workers use different proportions of the various ingredients. The writer has found the following method of preparing the medium to give the most satisfactory results.

Preparation of Water Agar.—In the first place ordinary water agar, containing sodium chloride, is prepared. The composition of this is:

Powder agar	..	..	15 to 20 grammes
Sodium chloride	..	..	8·5 grammes
Distilled water	..	..	1,000 cubic centimetres

This is put into a flask and boiled to dissolve the agar. It is advisable, but not absolutely necessary, to filter the agar. If it is to be filtered this will have to be done inside a 'steamer' or with a special water bath filter funnel while the agar is still hot. Cotton wool may be used for filtering if special filter paper is not available. We have found that 1·5 per cent. agar is sufficient in the cold weather in Calcutta, but that 2 per cent. is better for the hot weather. The agar is then decanted into previously sterilized test tubes; if three-quarter inch test tubes are used 6 c.c. should be put in each. It is important that the amount put into the test tubes should be approximately correct. The tubes are then autoclaved at 120°C. for twenty minutes. If they are to be used immediately they can now be transferred to a water bath and allowed to cool to 45°C., if not, they can be put aside and remelted when required. The medium is now ready for the addition of rabbit's blood. The most satisfactory means of obtaining this is by heart puncture.

Technique of Heart Puncture.—Special requirements:

A rabbit of not less than 4 lbs.

An animal operating board. Any flat board, 18 ins. by 30 ins., with a large nail at each corner, will do for this.

A 20 c.c. syringe with a medium-sized needle.

A spirit lamp, olive oil, etc.

The rabbit is tied on its back on to the table with four tapes, one looped round each limb and attached to each of the four nails. The fur on its chest is cropped and the skin sterilised. The giving of an anæsthetic is advocated by some. Ether should be

used. The writer does not consider that there is any advantage in giving an anæsthetic, as the principal discomfort that the animal suffers from is fright, and the administration of ether must be just as frightening as the cardiac puncture itself. The syringe should be oil sterilised (at 160°C.) and washed out with sterile citrate saline. The best place to puncture is about the third left interspace upwards from the xiphisternum, or the point slightly above the point of greatest cardiac impulse. The needle should be passed vertically to the skin surface with a slight inward (towards the middle line) tendency, and to a depth of about an inch. At the same time the plunger is withdrawn, and if the point of the needle is in the ventricle the blood will rush into the syringe. From this sized animal it is usually safe to withdraw 15 cubic centimetres of blood, although as much as 20 cubic centimetres can be withdrawn without killing it. About one minute should be taken to withdraw this amount. It is not advisable to withdraw blood from a rabbit in this way more than once in a fortnight.

Addition of Blood to Agar.—The agar must meanwhile be in a water bath kept at the constant temperature of 45°C. With aid of an assistant, or preferably two assistants, the plugs are rapidly withdrawn from the agar tubes and two cubic centimetres of blood run into each. The plugs are replaced after being 'flamed', and the contents well mixed by rapidly rolling the tubes in an upright position between the palms of the hands. Shaking the tubes should be avoided, as bubbles will be formed. The tubes are then sloped and allowed to cool. This procedure will have to be carried out very rapidly, as otherwise the blood will clot in the syringe. When the tubes have 'set' they are placed in a 37°C. incubator for 24 hours to test their sterility, after which they are ready for use. If the medium is not to be used immediately, it is advisable that the tops of the tubes should be covered with rubber caps in order to prevent evaporation of the condensation fluid.

Modification.—Young and van Sant (1923) suggested a modification of this method, depending on the fact that when blood is suspended in Locke's solution slow centrifugalization will throw down the red corpuscles but leave the leucocytes in suspension, and that rapid centrifugalization of the supernatant fluid will then throw down the leucocytes; these can be picked up with a sterile pipette and inoculated into N.N.N. medium. These writers also pointed out that the heavy parasitised cells fall to the bottom; if, therefore,

the lowest layer of cells be transferred to a slide, the parasites will be demonstrated more easily than by the examination of the direct blood smear.

CYTOLOGICAL CHANGES

There are a number of constant changes in the numbers of the various blood corpuscles and in their numerical proportion to one another which are of aid in the diagnosis of the disease and in gauging the amount of progress in a case under treatment.

The anæmia is of the pernicious rather than of the chlorotic type.

Erythrocytes and Hæmoglobin.—In a well-established case of the disease in an Indian the red blood corpuscles will number about 3,000,000 per c.mm. and the hæmoglobin percentage will be about 60. The hæmoglobin is estimated on the European standard, which is considerably higher than the Indian. An uncomplicated case does not usually show a greater degree of anæmia than this, but we have seen cases which were also heavily infected with ankylostoma with less than a million red cells per c.mm. Nucleated red cells are very frequently seen in blood films from kala-azar cases, as also are polychromatic staining red cells and red cells containing nuclear fragments.

The Leucocytes.—Most important and consistent changes occur here.

The Total Count.—The leucopenia which occurs, frequently as low as 2,000 per c.mm., is considered by some to be almost diagnostic of the disease. About 80 per cent. of well-established cases have a total white blood count of less than 4,000 per c.mm., but a leucocytosis does not exclude the possibility of the case being kala-azar, as in the presence of any septic complication the white blood count will be markedly increased and even in the absence of any obvious complication it is occasionally above 10,000 per c.mm. On the other hand, in non-leishmania cases simulating kala-azar clinically, a decided leucopenia may be present. An important diagnostic point in connection with the blood counts is the proportion of the red cells to the white. In Europeans the normal counts are 5,000,000 and 7,500 per c.mm., respectively, for the red and white cells, but the Indian has a normal leucocyte count of 6,000, and it is often as low as 5,000 in a normal individual. Thus the

normal proportion of red to white cells in a European is 666 to 1; in the kala-azar case the proportion is usually more than 1,000 to 1. This fact was looked upon by Rogers as an absolutely diagnostic point, but we can claim to have seen a number of cases in which the proportion was greater than this but which proved not to be cases of kala-azar. Nevertheless, if the accuracy of the blood count be relied upon, this degree of relative leucopenia should be counted very strongly in favour of a diagnosis of kala-azar.

Technique.—The writer's method of making a total blood count in a case which is suspected of being one of kala-azar is as follows:—An ordinary Thoma-Zeiss hæmocytometer slide cell and diluting pipette are used. The diluting fluid should contain one per cent. acetic acid with gentian violet as the colouring agent. The one-in-ten diluting pipette is used. The blood is taken, diluted, mixed, and placed on the slide in the ordinary way. A $\frac{1}{6}$th objective and a No. 2 eye-piece are used on the microscope. The slide is placed on the microscope and the squares are focussed. The tube is then drawn out until the diameter of the field measures exactly eight twentieths of a millimetre, that is to say, eight times the length of the side of a small square. The area of the field will now be (πr^2), or almost exactly equal to the area of 50 small squares. The length to which the tube has to be withdrawn should be noted, so that on all subsequent occasions the field of the right size can be obtained immediately by drawing out the tube to the required length. The count is made by counting the number of leucocytes in 40 different fields. The calculation is made in the ordinary way, remembering that each field contains 50 squares; for example if there are 125 corpuscles in 40 fields the count will be $\frac{4000 \times 10}{50 \times 40} \times 125$, or 2,500.

This method is particularly applicable in kala-azar. In the example given above, counting by the method which most people seem to adopt, that is, counting the contents of the 256 small squares, the total leucocytes observed would have been 16 only. Counting so small a number leaves a very big chance of error, and if more were to be counted the slide would have to be washed and prepared again. If the accuracy of the result is to be compared with that of the method we have mentioned above, this process would have to be repeated eight times. If there is no marked leucopenia it will only be necessary to count about 20 fields, but if by any chance there is a leucocytosis it will probably be

easier to count the contents of the 256 squares. The excuse for mentioning this method here is that so few people, in India particularly, appear to know it, and if any reliance is to be placed on blood counts an accurate method must be adopted.

Differential Count.—There is always a marked diminution in the polymorphonuclear leucocytes, the average count being about 30 per cent. or just under 1,000 per c.mm. The eosinophiles are markedly diminished, frequently none being found in a count of 250 corpuscles. As the eosinophiles are small in number normally this diminution will not be very obvious, but in the presence of a complication such as ankylostomiasis, where in the ordinary way you would find a distinct eosinophilia, it is a noticeable fact that this eosinophilia is absent. In a large number of kala-azar cases in which ankylostoma ova were found in the stool the average eosinophile count was 76·78 per c.mm., or 1·7 per cent., whereas for uncomplicated ankylostomiasis it was 2192·86 per c.mm., or 19·3 per cent. (McVail, 1922). The large mononuclears will be increased relatively, but actually they are not increased. There will also be a slight relative lymphocyte increase, but if the total number is calculated it will be found there is an actual decrease. As in the case of the total count, it is important that some standard method should be adopted for doing the differential counts. The writer (1922*b*) has found that the most accurate results can be obtained by making a very small film and counting all the corpuscles on that film.

The Typical Blood Count.—The typical blood count of a case of five months' standing is—

	Kala-Azar Case	Normal for an Indian
Hæmoglobin	60%	85-90%
R.B.C.	3,000,000 per c.mm.	5,000,000
W.B.C	2,500 ,, ,, ,,	6,000
Polymorphonuclears	30% or 750 ,, ,, ,,	70% or 4,200 per c.mm.
Small Lymphocytes	51% or 1,275 ,, ,, ,,	23% or 1,380 ,, ,, ,,
Large Mononuclears	18% or 450 ,, ,, ,,	5% or 300 ,, ,, ,,
Eosinophils . . .	1% or 25 ,, ,, ,,	2% or 120 ,, ,, ,,

BIO-CHEMICAL CHANGES IN THE BLOOD

Our knowledge of the bio-chemical changes in the blood in kala-azar patients is at present very incomplete, but we do know that certain very profound changes occur.

The Reaction of the Blood.—The alkalinity of the blood is said to be decreased (Rogers and Shorten, 1915). More recent work on the subject (Napier, 1924*a*) shows that, as one would naturally expect, the hydrogen-ion concentration of the blood in a patient who is not *in extremis* is practically unaltered. It is certainly true that a less amount of N/10 HCl is required to make any given quantity of the blood of a kala-azar patient neutral to litmus than is required to increase the hydrogen-ion concentration of the same amount of normal blood to the same degree (i.e. the neutral point for litmus), but it is also a fact that the addition of less N/10 NaOH is required to make a given quantity of kala-azar blood neutral to thymol-phthalein than is required to reduce the hydrogen-ion concentration of the same amount of normal blood to neutrality to this indicator. It might, therefore, be claimed that the acidity of the blood is also reduced in this disease. A more accurate way of stating this would be to say that in kala-azar the hydrogen-ion concentration of the blood is usually about normal, but that the stability of the hydrogen-ion concentration is decreased.

The Coagulability of the Blood.—In some cases the coagulation time is very considerably prolonged. It will be noticed that when the blood is taken for serum tests the corpuscles will occasionally settle to the bottom of the tube before the blood has coagulated. The writer has estimated the coagulation time in a number of cases and has found prolonged coagulation time not to be a constant feature of the disease, but that in some cases the coagulation was delayed for 5½ minutes. Wright's technique was used to estimate the coagulation time. Knowles (1920) found that the coagulation time varied from 2½ to 5¼ minutes, with a mean at $3{\cdot}28 \pm 0{\cdot}292$ minutes. The practical importance of these observations is that delayed coagulation is looked upon by some people as a contra-indication to spleen puncture. In actual practice we have not found this to be an important factor.

Calcium Content.—The calcium content was reduced in every case of kala-azar that has been tested at the Calcutta School of Tropical Medicine. The normal content for an Indian appears to be from 10·2 to 10·4 milligrammes per 100 cubic centimetres of serum; in more than half the kala-azar cases tested the content was reduced below 9 milligrammes, and in a few instances it was as low as 8 milligrammes.

The Blood Sugar.—A reduction in the blood sugar is a consistant finding in this disease. The normal blood sugar for an Indian is 0·1 per cent.; in kala-azar it is always below this figure, and occasionally it is as low as 0·05 per cent. The laevulose tolerance was tested in a number of the writer's cases and was found to be reduced; in most cases a rapid improvement in the tolerance was observed when the patient was given antimony injections.

The Globulin Content.—Sia (1921) states that the globulin content of the blood is increased in kala-azar, and Ray (1924) has shown that there is a very marked increase in the euglobulin element. Brahmachari (1920) reports that the globulin in the blood of a kala-azar patient possesses certain anti-complementary properties.

Anti-bodies.—Cornwall and LaFrenais (1916) were not able to demonstrate the presence of anti-bodies in the blood of a kala-azar patient, nor any antigenic properties in flagellate cultures; they concluded that the morbidity of the infection was dependent on direct cell destruction. DiCristina and Caronia (1913) claim that a complement deviation reaction, using extract of the organs of kala-azar patients as antigen, is of some diagnostic value in early cases of the Mediterranean type of the disease. More recently, Noguchi (1924) found that a serum with specific agglutinating properties can be produced by giving massive injections of flagellate cultures into the veins of rabbits. Dilutions of 1 in 100 gave marked agglutination with a culture of the homologous flagellate, and complete and rapid agglutination could be produced with a 1 in 10 dilution. He showed that *L. infantum* and *L. donovani* were almost identical, but that *L. tropica* and *L. brasiliensis* belonged to two different serological groups.

So far no satisfactory diagnostic agglutination test has been devised, but in view of this recent work it seems possible that one may eventually be devised; such a test would be of great value if it were applicable to the early stages of the infection.

SERUM TESTS FOR KALA-AZAR

The Globulin Precipitation Test.—This test was first suggested by Brahmachari (1917), who pointed out that if the serum of a kala-azar patient is added to excess of distilled water a copious precipitate will appear. He also found this phenomenon occurred occasionally in the serum of patients suffering from certain other

conditions, for example, phthisis, chronic malaria, etc., but suggested as a modification of this test that the serum be added one part to two parts of water, and he claimed that if this were done a kala-azar patient's serum gave a positive result, but not the serum from patients suffering from the other conditions mentioned above, although it does occur in the case of certain obscure conditions of enlargement of the spleen.

The test has not been found of any great value in this country, as a large percentage of malaria cases and cases of obscure splenic enlargement also give a positive reaction. Furthermore, the reaction is not usually positive in the early stages of the disease.

The Hæmolytic Test (Ray, 1921).—This test depends on the fact that when a drop of blood is added to distilled water, as in the estimation of hæmoglobin in the Gower's hæmoglobinometer, the hæmolysed blood is not clear, but remains cloudy as if hæmolysis were not complete. This appearance is not due to failure of hæmolysis or to any unusual insolubility of the stroma of the red blood cells, but to the precipitation of the globulin with the subsequent entanglement of the stroma of the erythrocytes, which are naturally insoluble in this precipitate. The test, therefore, is simply a modification of the globulin precipitation test. It has the advantage of being simpler than the other test and can easily be performed at the bedside.

Young (1923), working in China, thinks this a very useful test. He says that it is more delicate than the aldehyde reaction; this sensitiveness is a disadvantage in India where malaria is so common, but in China, in districts where there is no malaria, the contrary is the case. Sia (1924) has worked out a method of estimating the reaction quantitatively; he takes a measured amount of blood, 20 cubic millimetres—places it in 0·6 cubic centimetres of distilled water in a small test tube, and notes the rate of sedimentation. If the solution becomes cloudy within five minutes the case is considered to be one of kala-azar; in an advanced case of kala-azar complete sedimentation occurs within 15 minutes. The sedimentation rate decreases as the case progresses under treatment.

The Aldehyde Reaction.—(Napier, 1921, 1922 and 1923). About five cubic centimetres of blood is withdrawn from a vein and allowed to stand long enough for the serum to separate. One cubic centimetre of clear serum is then placed in a test tube, three inches by

half an inch preferably ; to this one drop of 30 per cent. formaldehyde, in the form of commercial formalin. is added ; the serum is immediately well shaken, and placed in a test tube rack at room temperature. The following results will be obtained.

(*a*) If the blood is from a case of well-established kala-azar, four to five months or more, the serum will immediately become viscid, within a minute or two will have 'set' so that the tube can be inverted without the serum being spilled, and become whitish and opalescent. Within three to twenty minutes, the time varying with different cases, the whole of the serum will have become absolutely solid and opaque, like serum coagulated by heat or the 'white' of a hard boiled egg. If the serum happens to be hæmoglobin-stained the coagulated serum will have a pink tinge, which will change to a chocolate brown after 24 hours. *This reaction may be taken as absolutely diagnostic of kala-azar.*

(*b*) If the blood is from a case of kala-azar of short duration, under three months, a milky whiteness will appear in the serum soon after the formalin has been added, but the serum will not become solid for some time. A certain amount of solidification may occur during the 24 hours, and the final result will be a partially opaque jelly-like substance, with a whiteness in the serum almost as if a little milk had been added. *This reaction must be classed as doubtful*, as it may be confused with the next type of reaction, although with a little experience it is not very difficult to distinguish between them.

(*c*) If the blood is from a case of malarial cachexia, and in some cases of malaria, phthisis, leprosy, syphilis and certain obscure cases of 'spleen fever' the serum will become solid so that the tube can be inverted, but will remain quite clear for some time. Later a certain degree of cloudiness may appear, but the serum will never become completely opaque. *This reaction must again be considered doubtful*, but if the case is one of longstanding fever with a spleen enlarged below the navel *it can safely be looked upon as a 'negative' result*, as, if a case of this description were one of kala-azar a definitely positive result could be expected.

(*d*) If the blood is from a normal person, or a patient suffering from most diseases except those mentioned above, the serum will remain fluid and clear almost indefinitely. *This will never occur in a case of kala-azar except in the very earliest stages.*

This test was introduced by the writer in June, 1921, and since its introduction it has been given a very extensive trial. Out of the first 91 kala-azar cases (confirmed by spleen puncture) in which the test was tried, 89 gave a definitely positive result. Whereas in only one out of 59 cases that were clinically suggestive of kala-azar, but in which Leishman-Donovan bodies were not found by spleen puncture, was the test 'positive'. It was also tried in 50 other cases which did not in any way resemble cases of kala-azar, and in none of these was a positive result obtained. The patient who gave a positive result with the aldehyde test, but in which no Leishman-Donovan bodies were found by spleen puncture, was almost certainly a case of kala-azar, as she subsequently improved considerably under treatment and went away entirely free from fever. A culture was not made in her case. This first series consisted of patients who had applied for treatment between June and October.

During the next few months, November to March, the results obtained with the aldehyde test were less satisfactory, the percentage of kala-azar cases which gave a negative result rising from the 2 per cent. of the first series to between 12 and 15 per cent. From April onwards, however, more accurate results were again obtained, and during the months, May, June, July and August, the percentage of kala-azar cases giving negative results with this test was again very low.

It has been shown that during the first three months, or so, of the disease this reaction is usually doubtful and in some cases negative. This infrequency of the occurrence of misleading results during the hot weather months, and the comparative frequency during the cold weather, accords with our observation that the large majority of cases commence their illness in the cold weather months, November to February.

SPLEEN PUNCTURE

Findings.—The condition and number of the parasites in the spleen vary in different patients, from time to time in the same patient without apparent cause, and in a patient under treatment according to the stage at which he has arrived. Some cases show a number of rosette forms, whereas others show none; again cases will be seen in which the parasites are undergoing rapid destruction,



the cytoplasm being ill-defined and the nucleus and parabasal only staining well; cases occur in which the parasites have temporarily disappeared from the spleen. The writer has done a second spleen puncture and found very large numbers of parasites in a patient in whom the first spleen puncture, only a week before, had been negative. On the other hand, in a fatal case it is quite usual to find no parasites at all in the spleen, removed immediately after death, although spleen puncture a few days before may have shown that at that time there were a large number of parasites present. If a spleen puncture is done when the course of treatment is well advanced the parasites, if any are found, will be of the degenerating type mentioned above.

Contra-indications.—Provided that you can have the patient under proper control and under observation for an hour or so after the operation, there are few contra-indications to the performing of spleen puncture. The writer has now performed this operation more than two thousand times without giving rise to any ill effects except temporary pain over the splenic area in a few instances. In cases in which the spleen is exceedingly soft it is as well to avoid the operation. It is said to be dangerous to puncture the spleens in cases of leukæmia; the writer has, however, done a number of such cases without any ill effects. The necessity for a spleen puncture in these cases can usually be avoided by the previous examination of a peripheral blood smear. Knowles (1920) reports a case of kala-azar in which the blood picture suggested spleno-medullary leukæmia, there being 75,000 white blood cells per c.mm., including numerous myelocytes, so that a diagnosis of the latter condition should not be ventured too readily. It is also said to be dangerous to perform spleen puncture in cases in which the coagulation time is very much prolonged. This, *per se*, is not sufficient reason for putting off a spleen puncture which is otherwise considered advisable. One would obviously not puncture the spleen of a hæmophilic.

Technique.—

The Preparation and After-treatment of the Patient.—If the patient is in hospital or under proper medical supervision, on the night before the operation he should be given calcium lactate, grs. xxx; this dose should be repeated first thing in the morning, and again after the operation. If the spleen puncture is to be done

at 10 a.m., the patient should have a glass of milk and a little bread at 7 o'clock, but nothing else. The splenic area should be sterilised with spirit or iodine, and the spot at which the skin puncture is to be made should be touched with pure carbolic acid on a match, a circle about a quarter of an inch in diameter being made. After the operation is completed and the puncture has been sealed with collodion, a pad should be placed over the splenic area and a tight roller bandage wound round the abdomen. The patient should be made to lie for an hour quite still; after this he may take his food. The object of the bandage is rather to limit the movements of the abdomen than to stop possible hæmorrhage by direct pressure. When necessity arises, it is quite justifiable to do a spleen puncture where circumstances are less ideal, but it should not be done unless the patient can be kept still for one hour after the operation. In the out-patient department of the Calcutta School of Tropical Medicine we give calcium lactate once only half an hour before the operation, we make the patient lie still for half an hour, and then sit in the waiting room for about another hour while the smear is being examined, after which time, if he is not showing any untoward symptoms, he is allowed to go home.

Requirements.—A five or ten cc. serum syringe, all-glass, 'Record' or Roux, but not all metal, with a new bright needle of medium calibre, and about an inch and a half long, is required. If a culture is to be made oil sterilisation will be necessary, but if direct smears only, it is advisable to have the needle only sterilised in boiling oil and the syringe itself sterilised and dried out with alcohol and ether. For the sake of the smears it is important that the syringe should be absolutely dry. If a culture is to be made a drop or two of citrate solution should be kept in the syringe. The slides and the N.N.N. medium should be placed on a table by the bed, and a spirit lamp should be burning.

The operation.—The patient should be made to lie flat on his back without a pillow, but with his hands underneath his head. The operator should stand on the left of the patient, and the assistant, whom it is always advisable to have—a nurse, if one is in a hospital—on the right. The most comfortable position for the operator will be found to be sitting on the edge of the bed. The spot to choose for puncture depends somewhat on the individual case, but usually the best place is about half an inch below the costal margin, at a

point half way between the anterior and posterior borders of the spleen. This point is sterilised as mentioned above. The assistant now places his right hand on the abdomen, so that his thumb comes to the right of and the fingers below the spleen. He does not exert any pressure, but simply prevents all downward movements of the organ. The puncture is performed in two movements. In the first place the skin only is punctured; this should be done by forcing the needle through the skin at a very oblique angle to the skin surface, so that the point does not go further than the subcutaneous tissue. Then by a second movement the needle is plunged into the spleen substance; this should be done at an angle of 45 degrees with the skin surface and in an upward and slightly outward direction, that is to say, in the direction of the long axis of the spleen, and to a depth of 1 to 1½ inches. The plunger of the syringe should now be rapidly withdrawn and released two or three times, and then the needle quickly withdrawn. (Plate XI.) If only a smear is required it will not be necessary to wait until blood or spleen pulp is seen in the syringe, as the minutest trace in the point of the needle is sufficient for this purpose, but if a culture is required it may be necessary to withdraw the plunger half a dozen times or more, until blood is seen in the barrel of the syringe mixed with the citrate solution. In the former case the needle should only remain in the spleen for a second or two; it is quite obvious that the longer the needle remains in the spleen the greater the danger of its tearing the capsule.

The contents of the syringe are squirted out on to a slide, and one or more smears are made. If a smear is required as well as a culture, it is advisable first to take one drop of spleen juice on to a slide from the syringe, avoiding contamination of the point of the needle, before this has been mixed with the citrate in the syringe, and then to inoculate the N.N.N. tubes with the spleen material which has been drawn into the barrel of the syringe. Occasionally blood will rush into the syringe directly the spleen is punctured; if this occurs it means that the point of the needle has punctured a venous sinus in the spleen substance. When this occurs the spleen puncture may be classed as unsatisfactory, as only blood and not spleen pulp will be obtained. It is rather surprising that even in an unsatisfactory puncture of this kind a very large number of parasites are often found.

The slides are stained in the same way as the blood films, and the culture tubes are placed in the cool incubator and treated in the same way as peripheral blood cultures, except that they can be examined at an earlier date; flagellates can usually be found in fair numbers about the fifth day.

The Diagnostic Value of Spleen Puncture.—It is probable that in about 90 per cent. of kala-azar cases parasites are found in the smears from the first spleen puncture (Napier, 1923*a*), but there are cases in which the third and even the fourth spleen puncture smears are 'negative', but in which flagellates are grown on culture. In partially treated cases it is usually impossible to demonstrate the presence of parasites except by cultural methods.

Liver Puncture

The Madras school favour the performance of liver puncture to that of spleen puncture. The strongest argument that they bring forward in favour of this operation is that the liver is usually tougher than the spleen, and it is, therefore, less likely to be torn. This is a very sound reason, as the liver in kala-azar is often exceptionally tough, whereas the spleen is exceptionally friable. The greatest argument against this operation is that it is not a certain means of making a diagnosis. The writer has on more than one occasion found a liver puncture 'negative' and on the same or following day found large numbers of Leishman-Donovan bodies in the material obtained from the spleen. A second point is that, if carefully conducted, spleen puncture is a perfectly safe operation and liver puncture is not altogether free from risk, as recently a fatality occurred in Madras after this operation.

Bone Puncture

As methods of diagnosis trephining the head of the tibia and sternum puncture have been suggested. There is little, if anything, to recommend these methods. In a case in which there was unbearable pain in the tibia, the head of the bone was trephined for the double purpose of making a diagnosis and of relieving the pain. The procedure was rewarded with double success. Sternum puncture was performed in the Carmichael Hospital for Tropical Diseases in three cases by an advocate of

this method. The operation seemed to be an extremely painful one, and in none of the three cases were Leishman-Donovan bodies found in the smears made from the material thus obtained. All three were cases of kala-azar, subsequently diagnosed by spleen puncture, and one showed leishmania in the peripheral blood.

THE URINE

As a rule there does not appear to be serious dysfunction of the kidneys. During the pyrexial periods the urine shows the usual characteristics associated with fever. In about 30 per cent. of all cases there is a trace of albumen in the urine but there is usually not more than a trace and there are seldom any casts present. There are frequently a few pus and desquamented epithelial cells.

Urobilin is present in a large percentage of cases. Knowles (1920) considers that the presence of urobilin associated with a trace of albumen in the urine constitutes a useful diagnostic point.

The chlorides are usually present in the normal quantities.

Shortt (1923*a*) obtained a flagellate growth from the urine of a patient suffering from kala-azar by placing the centrifugalised deposit from the urine in N.N.N. culture medium.

Knowles (1920) reported a case which terminated in acute nephritis with suppression of urine.

THE SKIN

The parasites are not present in any numbers in the skin tissues during the normal course of the disease; their presence in skin lesions, which has been noted from time to time, is probably accidental and consequent upon their presence in the peripheral blood.

POST-KALA-AZAR DERMAL LEISHMANIASIS

In the early, depigmented stage of this condition it is not usually possible to find any parasites by direct examination of the tissues, although cultural methods may show their presence.

In the nodular stage the parasites are usually present in large numbers.

Examination of the Lesions.—A nodule is gripped with a fine pair of forceps, and with a sharp pair of curved scissors a piece is snipped out. The under surface of this snip, which should be

about two millimetres thick, is smeared on a slide; the smear is then stained with either Leishman's or Giemsa's stain, and examined in the usual way for the presence of parasites. Sometimes large numbers will be seen immediately, but as a general rule a careful search will have to be made; the parasites usually lie in the endothelial cells, and are morphologically similar to the parasites found in spleen puncture smears.

If a culture is to be obtained the whole process will have to be carried out under strictly aseptic conditions. With a second pair of curved scissors a piece should be cut from the under surface of the snip, and dropped into an N.N.N. tube.

A new Serum Test for Kala-azar

Major R. N. Chopra, I.M.S., Professor of Pharmacology at the Calcutta School of Tropical Medicine, who has been carrying out some pharmacological tests with antimony compounds, recently drew my attention to the fact that when the serum of a kala-azar patient was added to a 4 per cent. solution of certain of the pentavalent compounds of antimony a very heavy precipitate appeared, but that when the same solution was added to the blood of a healthy individual no precipitate appeared.

I have now carried out this test with a number of pentavalent compounds in about fifty cases of kala-azar. My observations can be summarised as follows:

(i) The amount of precipitate formed by the addition of a definite kala-azar serum to different compounds of antimony is in proportion to the efficacy, according to my own experience, of that compound in the treatment of kala-azar.

(ii) The results obtained with the serum of kala-azar and non-kala-azar patients are almost exactly parallel to those of the aldehyde test; that is to say, little or no precipitate forms with the serum in the early stages of the diseases and the serum in some other diseases causes a certain degree of precipitation, but that otherwise the test can be relied upon as a reliable diagnostic method.

(iii) The reacting power of the serum develops if it is kept for 24 hours before the test is performed.

(iv) The reaction occurs with very weak solutions of some compounds, and with comparatively weak solutions clearer-cut results can be obtained.

As a technique I have found the following satisfactory:

To 2 cubic centimetres of a 0·25 per cent. solution of Stiburea in distilled water (or 0·1 per cent. solution of No. 693) add two drops (about 0·1 cc.) of 24-hour-old serum; agitate the tube to mix the contents. In a case of kala-azar a heavy flocculent precipitate will form, which within 15 minutes will settle, leaving a clear fluid above; if a fine precipitate forms which does not settle within this period, the result should be considered doubtful; with a normal serum the solution will remain absolutely clear.

Freshly separated serum will give equally good results, but with this it is advisable to use a 1 per cent solution of Stiburea (or a 0·25 per cent. solution of No. 693).

My experience with this test has been limited, but there are certain indications that clearer-cut results can be obtained than with the aldehyde test, and in cases in which the serum is milky—as is often the case with children's serum—the results with this test are quite clear, whereas the aldehyde reaction is clouded by the fact that the serum is already opaque.

1: 5: 27.

CHAPTER VI

DIAGNOSIS

Clinical diagnosis—Therapeutic tests—Laboratory methods of diagnosis—Differential diagnosis—Routine adopted in dealing with large numbers of patients.

IN this section the question will be treated from a practical point of view, the most important points being given in the order in which they are likely to come under one's notice rather than in the order of their relative importance.

CLINICAL DIAGNOSIS

Geographical and Family History.—A history of never having been in a known endemic area is naturally a strong point against the diagnosis of kala-azar, but it must be remembered that the extent of the distribution of the disease is not yet fully known.

A family history of the disease is a strong positive diagnostic point, but the absence of it is a very doubtful negative one. In a widespread endemic area, such as Bengal, we find that little help is obtained from family history, but in a more or less isolated community, such as a tea garden labour force in Assam, where the disease often takes an epidemic form, and where more exact information is available, if 'contacts' are regarded as potential cases of kala-azar, an early diagnosis can often be made.

History of Illness.—The most important points are:—(*a*) A history of long continued fever, resistant to quinine treatment, with a double daily rise; (*b*) progressive enlargement of the spleen; (*c*) progressive loss of weight; (*d*) a good appetite with bad digestion; (*e*) bleeding from the gums and epistaxis; (*f*) falling of the hair; and (*g*) increasing darkness of complexion.

Physical Signs.—The most important are:—(*a*) Emaciation; (*b*) dry, thin hair and harsh, rough skin; (*c*) characteristic pseudo-pigmentation; (*d*) visible pulsation of carotids in the neck; (*e*) spongy consistency of the enlarged spleen; (*f*) enlargement of the

liver; (*g*) comparative cleanliness of the tongue; and (*h*) a rapid pulse.

Discussion.—Diagnosis on clinical grounds only is seldom, if ever justifiable. It is not suggested that no aid can be obtained from clinical experience of the disease. An experienced clinician will make a correct diagnosis in nine cases out of ten, but when other aid is available it is not right to run the risk of condemning even a few cases of chronic malaria or of intestinal tuberculosis to a long, tedious and not altogether pleasant course of treatment, which will certainly do them no good, nor, on the other hand, seriously to imperil a number of kala-azar patients' lives by withholding from them a treatment which would with almost absolute certainty cure their disease. A typical kala-azar patient has a certain characteristic appearance which it is almost as impossible to mistake, when one is familiar with it, as it is difficult to describe on paper. Unfortunately, the typical case is rather the exception than the rule. The failure of the clinicians, in the days when the clinical science was not dulled by continual reliance on the microscope and when the famous physician did not step in fear of giving an opinion contrary to the damning evidence of a subsequent 'positive' laboratory report, to differentiate between this disease and chronic malaria in most of the areas of its greatest sporadic activity until the causative organism was discovered, demonstrates very clearly the difficulties with which the pure clinician is faced in distinguishing between these two conditions, and should encourage him to pocket his pride and seek the help of his less artistic, if more scientific, colleague, the pathologist.

The importance of making an absolutely definite diagnosis before treatment is commenced cannot be over-emphasized. One should aim at making a diagnosis which is not only absolutely convincing at the time, but which one will not doubt even after the patient has had 60 injections of sodium antimony tartrate without showing much sign of improvement. Nothing is so disastrous to the prospects of a patient as the existence of doubt as to the correctness of the original diagnosis in his medical adviser's mind. Furthermore, once the treatment has been commenced the difficulties of diagnosis are considerably increased.

THERAPEUTIC TESTS

Exclusion of the possibility of the disease being malaria by the administration of quinine or one of the cinchona alkaloids as an aid to clinical diagnosis is, under certain circumstances, such as the absence of a microscope, justifiable, but it must be remembered, on the one hand, that a large percentage of kala-azar cases will show a certain amount of improvement under treatment with this drug, and, on the other hand, that there is a difference between prescribing quinine and knowing that the amount prescribed has been absorbed; the honesty of the dispenser, the prejudices of the patient and the vagaries of his gastric mucosa have to be taken into consideration.

Treatment with sodium antimony tartrate before a definite diagnosis has been made is never under any circumstances justifiable. One can say, with a tolerable degree of certainty of being correct, that a fever which does not react to 20 grains daily (in two doses) of quinine or cinchona within seven days is not malarial in origin, but one cannot say that a fever which does not react to as many as thirty injections of antimony tartrate is not kala-azar.

Although under no circumstances is it good practice to give treatment for kala-azar as a therapeutic test, the introduction of the pentavalent compounds has opened up new possibilities, and, alas, new temptations. Five injections of many of these compounds will as a rule reduce the fever in a case of kala-azar. There are certain cases in which, other diseases—such as malaria and typhoid—having been excluded and circumstances not allowing a spleen puncture, it may be permissible to give a course of injections of one of these compounds, but once the decision has been made a full course of treatment should be insisted upon. Unfortunately, it is becoming a common practice in Calcutta to give, almost on sight, to any patient suffering from fever 'just a few doses of urea-stibamine and a quinine mixture'. If the patient happens to be suffering from one of the many fevers of short duration that are encountered in this city, or from malaria, the fever will disappear after about the third injection, the patient will be told that he has had kala-azar, and he may, or may not, be subjected to a proper course of treatment for this disease. On the other hand, if the fever continues despite about five injections, as is quite frequently the case in kala-azar, the treatment will probably be discontinued. Thus, whether the

patient is, or is not, suffering from kala-azar, he runs a very serious risk of getting the wrong treatment and the difficulty of arriving at a correct diagnosis is very considerably increased.

LABORATORY METHODS OF DIAGNOSIS

Under this heading the less elaborate bedside 'laboratory methods' are included.

The Aldehyde Test.—The aldehyde test is the one from which most valuable information can be gathered and is the easiest to perform. Should this reaction be strongly positive no further confirmation is required; on the other hand, should it be quite negative in a case that has a definite history of over six months' fever and a spleen reaching below the level of the umbilicus, a definitely negative diagnosis can be made. If it is only partially positive or doubtful, then further investigations will be necessary.

The Blood Count.—Very considerable reliance is placed by some observers on the blood picture. Rogers was of opinion that a leucopenia where the proportion of white to red corpuscles was 1 to 1,000, or more, was diagnostic of the disease, but we have often found this degree of proportionate leucopenia in cases of chronic enlargement of the spleen and anæmia unassociated with leishmaniasis. A total white count of less than 2,000 is, however, a diagnostic point strongly in favour of kala-azar. In a differential blood count, done by a reliable method, a decrease of polymorphonuclear leucocytes below 600 per c.mm., and also the absence of eosinophiles, especially if the latter occurs in conjunction with ankylostomiasis infection, are points of considerable diagnostic value.

Examination of the Blood Films for Parasites.—About half a dozen blood films should be taken and a careful search made for the parasites along the leucocyte edge. Here again, if one is fortunate enough to get a positive result further trouble will be saved, but in this case a negative finding is of little importance.

Spleen Puncture.—Finally a spleen puncture should be done, and at the same time a culture of spleen pulp should be made on N.N.N. medium. A satisfactory spleen puncture culture that has remained sterile for a fortnight may be regarded as negative and the case in question diagnosed accordingly. When other points are in favour of kala-azar, a single spleen puncture with microscopic examination of the slide alone should not be taken as final. The

writer has done as many as four punctures and has failed to find Leishman-Donovan bodies in a case in which the spleen puncture culture was positive. This is the usual experience in the case of leishmaniasis of experimental animals.

In the absence of splenic enlargement, liver puncture and culture should be carried out.

Blood Culture.—If there is still any doubt about the diagnosis and if the patient refuses to have a spleen puncture performed, or there is some contra-indication to this operation, a blood culture by Row's method should be done.

DIFFERENTIAL DIAGNOSIS

The main conditions from which the disease has to be differentiated are :—in the early stages, typhoid; in the later stages, malaria, tuberculosis (of all kinds), and other conditions in which long-continued fever is the main symptom, infantile cirrhosis of the liver, liver abscess, spleno-medullary leukæmia and other conditions (non-tropical) of enlargement of the spleen, and lastly, 'tropical splenomegaly'.

There is also a danger of one of the commoner complications being looked upon as the primary disease.

Typhoid.—Absence of characteristic drowsiness, the cleanliness of the tongue, the rapid pulse, the double daily rise of temperature and the remittent or intermittent nature of the fever, contrasted with the high continuous chart of a typhoid case, are the principal features which distinguish the two diseases. The difficulty of distinguishing between kala-azar in its earliest stages and typhoid or paratyphoid is illustrated by the fact that a large percentage of cases that come to the School of Tropical Medicine for treatment, in the month of June, give a very definite history of having been treated for 'typhoid' in one of the large Calcutta hospitals during the previous cold weather.

In a district in which kala-azar is endemic an attack of 'typhoid' should be regarded with the utmost suspicion, and in the absence of positive bacteriological evidence of the latter disease every effort should be made to exclude the former. This is not very easy. The blood picture of kala-azar at this early stage is not always typical. There may be a decided leucopenia, but there would also probably be a leucopenia in the case of typhoid.

At this stage also the aldehyde test will be negative. The Widal reaction, if this shows signs of increasing positivity, may be of some assistance, but a single test will not help. One is left with no alternative but a peripheral blood culture or a spleen puncture.

If an attack of typhoid is to be regarded with suspicion, then a relapse of typhoid is to be taken much more seriously, and if this occurs the failure to exclude the possibility of kala-azar by one of the two above-mentioned methods is inexcusable. Even bacteriological evidence of the presence of the typhoid bacillus should not be taken as final, as the writer had a case in which the patient suffering from kala-azar was also a typhoid carrier.

Malaria. — *Acute Malaria.* — The characteristic paroxysmal nature of the attack, the rigor, which is sometimes present in kala-azar, but which is not the typical malarial 'ague' and is usually not followed by sweating, and the complete reaction to suitable doses of quinine in malarial fevers as contrasted with the double daily remittent fever, the fact that the patient is often unconscious of the fever when it is as high as 103°, and the absence of reaction to quinine in the kala-azar case, serve to distinguish the two conditions.

Malarial Cachexia.—The paroxysmal nature of the malarial attack may not be so well marked, but the other differentiating points mentioned above hold good. In addition the soft consistency of the spleen against the hard 'ague cake' of malaria, the history of steady increase in size of the kala-azar spleen against the history of enlargement during attacks and decrease during the remission period, the length of history, seldom more than two years, and the rapid pulse rate of kala-azar are all distinguishing points.

The Blood Picture.—The finding of the causative parasite of either disease clinches the diagnosis of that particular disease, but does not exclude the possibility of the presence of the other or of the parasite of the other. A leucopenia will usually be present in malaria, but an extreme leucopenia of under 2,000 without an equal decrease in the red cells is a point in favour of kala-azar. The relative decrease in polymorphonuclears and eosinophiles are also contributory points in favour of the same diagnosis. An extreme general anæmia is more common in malaria than in kala-azar.

The Aldehyde Reaction.—Solidification and sometimes a slight

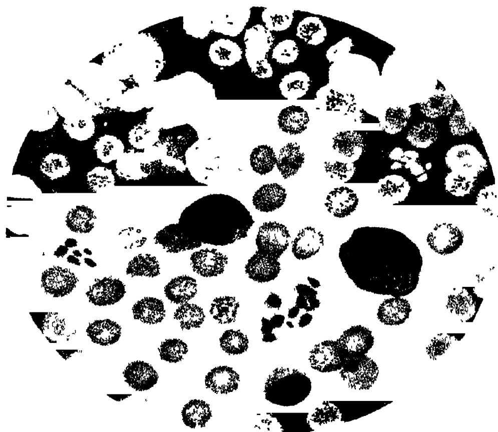


Plate XIV.

Spleen puncture smear of patient suffering from malaria.

degree of opacity occur in malaria, but unless the kala-azar is of recent origin this test will in most cases settle the diagnosis.

Spleen Puncture.—It may be necessary to resort to this measure if the diagnosis is still uncertain. Malarial parasites, in the form in which they are seen in the peripheral blood, will seldom be seen in the spleen material, but the presence of a large amount of malarial pigment, and occasionally of crescents, naturally leads to the diagnosis of this disease, but it does not exclude the possibility of co-existent kala-azar.

Other Fevers.—Relapsing fever and Malta fever do not occur in Bengal, Bihar and Assam, and the distribution of the former in the Madras Presidency does not coincide with that of kala-azar. Marked splenic enlargement is not common in either condition and there is usually some leucocytosis, especially in the latter. The finding of the spirillum in the peripheral blood of the former, and a positive serum test in the latter, should settle the diagnosis.

Tuberculosis, in association with enlargement of the spleen in any form in which the physical signs are not obvious, may give rise to considerable difficulty in diagnosis. The blood examination does not always help much, as a leucocytosis is by no means consistently present, but a definitely positive aldehyde reaction will decide the question. Obviously, the finding of the tubercle bacillus in the sputum, fæces, etc., would also settle the diagnosis.

Other Conditions of Enlargement of Liver and Spleen.—These are being dealt with together, as the well-recognised conditions that one might meet are so numerous, and so many of the cases which one does see do not fit in with any previously described condition. In few of these conditions is fever continuous. The patients will usually first come with fever, which, though the determining cause of their applying for treatment, is purely incidental. It seldom lasts for long, and the patient may be entirely free from fever for months at a time. In these conditions ascites is comparatively common, whereas in kala-azar it is rare. There may be gross changes in the blood picture which distinguish the disease from kala-azar, but, on the other hand, a blood count which would be described as typical of kala-azar is sometimes seen. A massive hard spleen will more frequently be seen in non-leishmania cases. It is in these cases that the aldehyde test gives very satisfactory results.

(*Specimen Case Card. Face.*)

CALCUTTA SCHOOL OF TROPICAL MEDICINE AND HYGIENE
KALA-AZAR CASE CARD

No. *1704.* Name: *Rajendra Kumar Das.* Disease: *Kala-azar.*

Date of onset: *Nov., 1922.*
Nature of onset: '*Enteric fever.*'

Fever:
- Periodicity at first: ***High remittent.***
- Periodicity now: *Low irregular.*
- Effect of Quinine: *Temporary only.*
- Rigors: *No.*
- Duration: *6 Months.*

Appetite: *Good.* Digestion: *Fair.*
Diarrhœa: *Occasional attacks.*
Dysentery —
Epistaxis + Bleeding from gums +
Falling of hair +
Lung symptoms: *Irritating cough.*
Loss of weight + Enlarged spleen +
Change of colour: *darker.* Palpitation +
Previous treatment: *Quinine and patent medicines only.*

Nutrition: Emaciated, thin, normal.
×
Anæmia +
Skin: Pigmentation +
Roughness —
Hair: *Thin.*
Tibiæ: Pitting +
Shininess —
Pulsation of Carotids +
Congestion of abd. veins +
Ascites — Jaundice —
Tongue: *Clean.*
Teeth and gums: *Healthy.* Pulse rate: *110.*
Weight: *4 st., 9 lbs.* Temperature: *101–102.*
Heart: *Hæmic murmur.*
Lungs: *Nil.*

	Liver.	Spleen.
Size:	*2 inch.*	*6 inch.*
Consistency:	*Firm.*	*Soft.*
Tenderness:	—	—

Provisional diagnosis: *Kala-azar.*

(Specimen Case Card. Reverse.)

No. *1704.* Name: *Rajendra Kumar Das.* Disease: *Kala-azar.*

Date: *May 14th, 1923.* Sex and Religion: *M. H.* Age: *10.*

Occupation: *Student.*

Present address: *4123, College Street, Calcutta.*
How long resident at this address? *One day.*

Native of: *Gopalpur, 24 Perganas.*
When last in native place? *Until a few days ago.*

Other places visited: *Nil.*
Family history: *Sister had kala-azar.*

Clinical diagnosis: *Kala-azar.*
Aldehyde reaction: + + +
Other peripheral blood findings: *W.B.C. 3,400.*
Spleen puncture findings: *L.D. bodies.*
Diagnosis: *Kala-azar.*
Whether expected to attend for treatment: *Yes.*

Treatment

Nature of treatment: *Sodium Ant. tartrate.*
Number of injections: *35.*
Period of treatment: *4 Months.*
Total amount of sb. given: *2·45 grammes.*

Result

Clinical: *Cured; now well; weight* + +
Spleen: *Only just palpable.*
Blood picture: *W.B.C. 9,500 per c.mm.*
Aldehyde reaction (+)
Date of discharge: *10th Sept., 1923.*

ROUTINE ADOPTED IN DEALING WITH LARGE NUMBERS OF PATIENTS

In the out-patient department of the Calcutta School of Tropical Medicine, where sometimes as many as 30 cases of suspected kala-azar have to be dealt with in one morning, the following is our routine. First the histories of the cases are taken on the kala-azar enquiry forms; then five cubic centimetres of blood is taken from a vein and placed in a test-tube in a sloping position; this can be done by an assistant.

The cases are next examined and a clinical diagnosis made.

The serum usually separates within half an hour. As each sample of blood becomes ready the serum is poured off into another test tube in the rack, and one or more drops of formalin added in proportion to the amount of serum. Within another 15 minutes it is usually possible to tell what the result of the test is going to be. If the result is definitely positive a diagnosis of kala-azar is entered on the patient's card and he is treated accordingly; if the reaction is negative and the patient has a very much enlarged spleen and a history of long illness, a diagnosis of 'Splenomegaly, not kala-azar' is made, and the patient sent elsewhere for treatment; but if the reaction is doubtful or negative and the patient's condition and history suggest that it may be a comparatively early case of kala-azar, a thick film is taken, the patient is given a prescription containing cinchona febrifuge, and is told to return in three days' time. The thick film is examined at leisure for malarial parasites. If malarial parasites are found, or if the mixture appears to control the fever, the patient is advised to continue to take the mixture and to come again if there is a return of fever. But if no parasites are found and the fever is not controlled a spleen puncture is performed. By adopting this routine, with the help of one assistant, 25 new cases—the average morning's work—can be diagnosed in about two hours.

CHAPTER VII

PROPHYLAXIS

Provincial measures—Prophylaxis on tea estates—In towns—Special measures suggested on the assumption that the sandfly is the transmitter.

In the absence of any exact knowledge of the means by which the infecting organism is transmitted it is impossible to lay down any hard and fast rules as to the measures that should be adopted to prevent the spread of the disease. There are, however, certain general lines of prophylaxis based on our knowledge of the epidemiology of the disease which are worth recording.

PROVINCIAL MEASURES

In Assam, where for a number of years a serious effort has been made to combat the disease, the two lines of attack that have been adopted have been segregation of the sick and contacts and treatment of the sick. Legislative measures have been introduced which make it possible for the health authorities to enforce segregation and to compel the sick to undergo treatment. Large numbers of dispensaries have been opened in various parts of the province for the treatment of kala-azar only, and specially trained doctors have been placed in charge of these dispensaries. The latest returns show that, out of a total population of 6,852,242, during the year 1925 60,940 persons were treated for kala-azar; it has been estimated that this number represents about half the number actually suffering from the disease. This reduction in what is probably the source of infection must, it seems, eventually lead to a reduction of the disease in the province. The eventual success of this policy will depend on how long it is continued. The next few years will possibly see a steady decline in the kala-azar incidence in the province—as a natural sequence of events after a period of high incidence, combined with the results of extensive treatment; if the present treatment policy is carried on into the quiescent years, then it seems very probable that a permanent reduction of the incidence of the disease throughout the province may be achieved.

In these days of successful treatment of the disease segregation is seldom resorted to, but in the days before the introduction of antimony treatment segregation measures were often the only hope of saving a community from almost complete annihilation. These measures were voluntarily undertaken by the villagers without even the advice of the health authorities; the patient, together with the rest of the family, were expelled from the village. Another method which was adopted by the Assamese was the removal of the whole village to a fresh site.

PROPHYLAXIS ON TEA ESTATES

The measures adopted for the check of the spread of kala-azar in a coolie community that have met with most success have been, shortly, the following: When a number of cases have occurred in one 'line' that whole line is looked upon as infected, and measures taken to abandon it. All members of uninfected houses are removed to a temporary 'contact line' and after six months' freedom from symptoms to a permanent new line, which should be as far away as possible from the old line, and certainly not less than 300 yards from it. All persons from infected houses that have shown no symptoms of the disease are, as far as possible, also moved into the contact line, but are kept under stricter observation and after a longer period are drafted into the new line.

In our ignorance of the exact means by which the disease is spread this method is by way of being a compromise. It is an attempt to leave behind both the source of infection—the patient—and the vector. If we felt sure that the vector would not be transferred, or would not already be present in the new line, then it would be unnecessary to differentiate between the sick, the contact and the rest of the population; they could all be removed to the new line, and only those already infected would suffer from the disease. On the other hand, if we could be certain of weeding out all the infected persons—presumably the source of infection—it would then be unnecessary to transfer the lines. This measure has been attempted many times in the past, but has never been successful; even when the contacts have been removed together with the sick, the progress of the disease amongst the remaining populaton has been apparently unchecked. On the other hand, the transfer of the lines is reported to have checked an epidemic on a number

of occasions (Napier and Foster, 1927). There is, of course, the danger that in each of the cases the transfer may have been delayed until the epidemic had burnt itself out, and that the effect of the transfer was only an apparent one.

Recently, the tendency has been to rely entirely on treatment and not to transfer the lines. In a few gardens where the early cases were treated and the coolies not transferred to new lines, it has been claimed that the epidemic was cut short; the introduction of the disease does not, however, always lead to the incidence of the disease on an epidemic scale, and there is no reason to suppose that an epidemic was threatened in the gardens referred to above. If one could be certain in every case of commencing treatment at the onset, then treatment should prove a very effective way of stopping the further spread of the disease, but, unfortunately, this is not possible. The average duration of the disease when the Bengal villager applies for treatment, even when this treatment is close at hand, is nearly six months, and in the case of the coolie who is under careful medical supervision it is doubtful if the average is much less than three months.

Treatment of the sick without transfer to new lines is in effect the same as segregation of the sick without segregation of the contacts, and as a preventive measure cannot be expected to be any more successful. There is just one point in favour of treatment over segregation; the promise of treatment will encourage the coolies to produce their sick, whereas the threat of segregation would encourage their concealment.

There is little doubt, both on theoretical grounds and from practical experience, that the early transfer of the lines to a fresh site, combined with early treatment of the sick, will produce the best results.

Whether or not from an economical point of view it is advisable to transfer lines is another matter. In these days of successful treatment humanitarian considerations do not come into account. The cure rate is very high, at least 95 per cent. if one of the pentavalent compounds is used, and sequelæ are negligible. It becomes a question of whether the prospect of the treatment of a large number of patients should be faced, or the coolies transferred to new lines. Much will depend on how soon this transfer can take place; if this can be done immediately it would probably be the better course to take.

One other point of importance is the time of year at which the transfer takes place. It seems probable that most infections occur during the monsoon period, so that every effort should be made to complete the transfer of the lines *before* the monsoon. The effect of removal immediately after the monsoon will be that the cold weather crop of cases, infected during the previous monsoon, will make their first appearance in the new lines. Even assuming that the vector is not present in the new lines the appearance of a large number of fresh cases will not tend to impress the coolies with the success of this measure.

IN TOWNS

The conditions under which the disease occurs in Calcutta have been very carefully studied; avoidance of these conditions, a summary of which has been given above, is the most important prophylactic measure to be taken. It may not always be possible to avoid living on the ground floor, but if there are a number of children in the household an effort should be made to do so; the rooms should be made as airy as possible, by the avoidance of overcrowding with furniture and by removal of all superfluous curtains; the verandah should not be choked with plants; care should be taken that there are no holes in the cement floors where earth and rubbish can accumulate; an area at least one yard wide around the house should be cemented, or at least paved; and ducks and fowls, if kept at all, should be banished to the most distant parts of the compound, and should on no account be allowed to roam freely in the compound, more especially into the ventilation space under the building.

It is difficult to suggest any precautions that can be taken by the hut-dweller in the town; it was, however, observed that the huts tightly jambed together in the most thickly populated parts of the town, with no compound and a paved courtyard, though thoroughly inhygienic from most points of view, were comparatively free from the disease.

SPECIAL MEASURES SUGGESTED ON THE ASSUMPTION THAT THE SANDFLY IS THE TRANSMITTER

The efforts of the research worker have been concentrated in attempting to prove that the sandfly is the transmitter of the



PLATE XV

A kala-azar infected hut in a Bengal village. Both the patients in the foreground contracted kala-azar whilst living in this hut, the arrow points to the opening of the fowl pen.

disease, and up to the present no effort has been made to work out any anti-sandfly measures suitable for the endemic areas, and, more especially, for the rural areas, the population of which provide 99 per cent. of the kala-azar cases in India. The suggestions made above could be described as anti-sandfly measures, but they are only suited to the town-dwellers and could not be adapted to the rural areas.

The thin-walled bamboo, or *ekra*, hut should certainly provide the sandfly with less protection against climatic changes than does the thick-walled mud hut. Argentipes are less frequently found in the hut that has a carefully plastered floor. The purely theoretical suggestion made by the writer (Lloyd, Napier and Smith, 1925, and Napier and Smith, 1925), that the relative position of the chicken house, the cowshed and the sleeping quarters may prove an important point in village sanitation, has not yet been investigated practically, but the habit of keeping the chickens in a hole underneath the hut itself is obviously to be discouraged. We know that the sandfly prefers human to avian blood and bovine to human blood, but we do not know whether the closer proximity of a cow would tend to attract more sandflies to the sleeping quarters, or would provide a more attractive meal for the few that were already there.

The sandfly, *P. argentipes*, is very sensitive to smoke and is seldom found in a room where the family cooking is done. Periodic fumigation of the sleeping quarters, with either sulphur, cresol, or crude tobacco would probably kill the adult flies which had remained in the room, and nightly fumigation with something less ohnoxious, such as *dhuna* or *lobung*,[1] might discourage their entry. The use of fine muslin nets in the place of ordinary mosquito net, when possible, is to be recommended. Another line of attack would be an attempt to destroy the larvæ by periodically spraying the possible breeding grounds with some antiseptic, or by attempting to make the soil around the hut unsuitable by the addition of some cheap chemical, such as lime; as the range of the sandfly is a very short one, and as they do not apparently breed out in the open, the area that would have to be treated is not a wide one. General tidiness and cleanliness of the courtyard would certainly tend to the reduction of suitable breeding places.

[1] Two kinds of resin used in India for driving away mosquitoes.

These suggestions are all tentative ones and not based on practical experience, as up to the present the sandflies of Bengal and Assam have not been looked upon as dangerous, or even as a pest. The measures that have been adopted in other parts of India and in other countries are obviously unsuited to the conditions in these two provinces. If the sandfly is proved to be the transmitter, a new field for research will be immediately opened up.

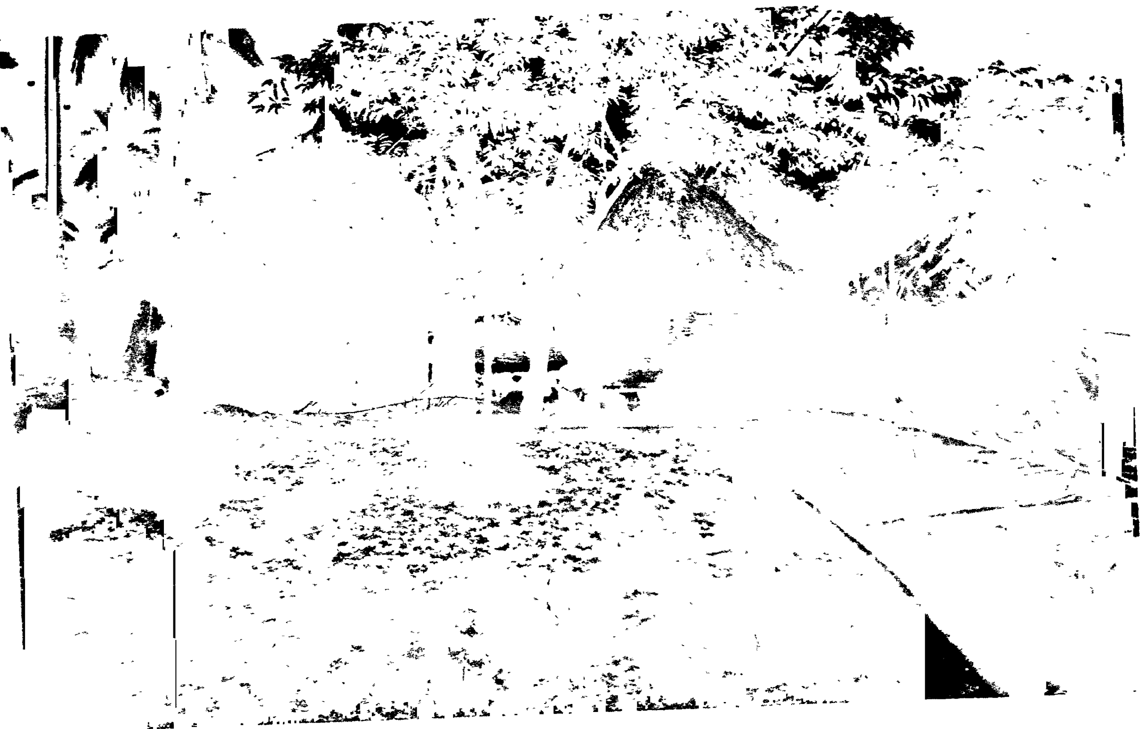


PLATE XVI

CHAPTER VIII

TREATMENT

Introduction of the specific treatment—Methods of administration—Various antimony preparations—Antimony salts of organic acids—The pentavalent compounds of antimony—Pharmacological action of the various preparations—Treatment by the antimony tartrates—Treatment by the pentavalent compounds—Stibosan—Urea-stibamine—Stiburea—Stibamine glucoside—Aminostiburea—Von Heyden 693—Complications due to treatment—Relapses—Treatment by intramuscular injections—Subsidiary treatment—Diet—Treatment of complications—Prognosis—Treatment of dermal leishmaniasis—Summary of treatment.

History of the Introduction of the Specific Treatment

Before the introduction of the treatment by intravenous injection of tartar emetic the course of the disease was practically unaffected by treatment. Cures were claimed by different observers, who used such various remedies as huge doses of quinine, intramuscularly, hypodermically and by the mouth, streptococcal vaccine, ductless gland extracts, killed cultures of flagellates, turpentine injections, etc. The percentage of cures claimed by the most enthusiastic was never very large, and was probably not much greater than the spontaneous cure rate. These natural cures have been placed as high as 25 per cent. by some observers. A severe inflammatory complication, such as pneumonia, is frequently followed by complete cure. It was this fact that led to the use of drugs such as turpentine, which produce an artificial leucocytosis and were probably the most successful of all the earlier forms of treatment.

Intravenous tartar emetic was first used by Martin and Leboeuf (1908) in the treatment of trypanosomiasis, and subsequently by Vianna and Machado (1913), in the treatment of American mucocutaneous leishmaniasis.

Caronia and Di Cristina (1915) treated a number of cases of infantile kala-azar in Italy with intravenous injections of tartar emetic with singular success. They were followed immediately by Rogers (1915) and Muir (1915), who had similar successes in the

treatment of kala-azar in this country; later Knowles (1920) treated a number of Assam cases at Shillong, checking the results by spleen puncture and culture.

For the next five years little progress was made in the treatment of the disease as far as India was concerned; the sodium salt was substituted for the potassium, and a number of chemical manufacturers put specially purified brands of this salt on the market, so that the severe reactions which were inseparable from the use of the less pure potassium salt became rare.

THE INTRODUCTION OF THE PENTAVALENT COMPOUNDS OF ANTIMONY

During this period the firm of von Heyden introduced a pentavalent aromatic compound, sodium para-acetyl-amino-phenyl-stibiate; this compound was used in Italy by Caronia (1916) and later by Spagnolio (1920) in the treatment of infantile kala-azar. Their results were promising.

Manson-Bahr (1920) treated one case of kala-azar in England with this compound, and reported favourably on it. In the same year Mackie (1921) obtained supplies from Messrs. Allen and Hanbury, through whom it had been placed on the market under the trade name Stibenyl. His results were most discouraging, as also were those of other workers in this country. The present writer (1922*c*) submitted a paper, which was read at a meeting of the tropical diseases section of the Royal Society of Medicine in which the results of ten cases treated with this compound were reported; the results were far from encouraging, and it was suggested that this compound might be unsuitable for export to a tropical country. Brahmachari (1922*a*), who had been working with the aid of a grant from the Indian Research Fund Association for some time on antimony compounds, produced a substance which he named urea-stibamine, a combination of urea with para-amino-phenyl-stibinic acid; he reported the results of the treatment of eight cases. These results appeared promising, but as the details were rather meagre most observers were unconvinced at that time that any great advance had been made. Shortt and Sen (1923), working at Shillong, also under the Indian Research Fund Association, gave the compound a full trial and reported very favourably thereon. In the same year the present writer (1923*d*), who had been independently

testing a number of antimony compounds, reported very favourably on meta-chlor-para-acetyl-amino-phenyl stibiate of sodium, a compound which he had used in the treatment in eleven cases; later (1925*a*) he published the results of the treatment in ten cases with stibamine glucoside and seven cases with aminostiburea (1925*c*), both pentavalent compounds of antimony of great therapeutic value, which had not previously been used in the treatment of leishmaniasis. Finally, the present writer (1927) published a paper, based on a long series of cases treated with No. 693, another von Heyden preparation, in which he claimed that this was the most powerful antimonial that had been used in the treatment of kala-azar.

METHODS OF ADMINISTRATION

Since the specific action of antimony in this disease was demonstrated, all possible methods of administration have been investigated, but the intravenous route is the only one for which uniform success can be claimed; all the compounds which are in use at present for treatment of the disease can be introduced in suitable strengths directly into the venous blood stream with absolute safety. The only possible objection to this method of administration is that a certain degree of special technical skill in the administrator is essential. A few years ago this appeared to the writer to be a very real difficulty, but now in Bengal even the members of the lowest grade of qualified practitioners have all become adepts at intravenous therapy.

Under certain circumstances intramuscular injections are a comparatively good substitute for intravenous injections, but no advantages can be claimed for subcutaneous administration, as this almost invariably produces cellulitis, which may lead to abscess formation.

Smyly (1927) has given intraperitoneal injections in a few cases with success. He gave the usual intravenous dose of sodium antimony tartrate diluted in 50 cubic centimetres of normal saline. This method can only be adopted with safety under hospital conditions, but might prove useful in the rare instances in which it is not possible to find a suitable vein.

None of the compounds that have been produced up to the present are suitable for oral administration. The tartrates are very irritant if taken in anything but minute doses, and the pentavalent

compounds are apparently not absorbed. The writer gave two patients one gramme of Stibenyl a day for fourteen days without obtaining any clinical improvement in either case; the urine at no time contained more than a trace of antimony, not more than would have been found had the patients been receiving 0·02 gramme intravenously on alternate days. It is obvious that this is the ideal method of administration if only a suitable compound could be produced.

Other methods of administration, such as rectal, by inunction and by ionization, have been tried without success; even had they been successful they are not methods that could be adopted for general use.

The Various Antimony Preparations

The various preparations that have been used in the treatment of kala-azar can be conveniently divided into three groups:

(1) The antimony salts of organic acids (trivalent antimony).
(2) The pentavalent compounds of antimony.
(3) Other forms of antimony.

1. ANTIMONY SALTS OF ORGANIC ACIDS

(*a*) Tartar emetic, or potassium antimony tartrate, was the first form of antimony to be used with success in the treatment of the disease; some practitioners still prefer this salt to the sodium salt.

(*b*) Sodium antimony tartrate is the salt which is most generally used. It is less toxic than the potassium salt and is more soluble. The greater solubility is a distinct advantage, as it makes it possible to prepare a scale form of the salt. A certain degree of chemical purity is essential for the preparation of this scale form, and as the purity of the salt is an important point the use of the scale form gives the practitioner a feeling of security. Since the writer (1922) pointed out the advantage of this scale preparation, a number of manufacturing firms are preparing this form of the salt almost exclusively.

A scale preparation, which was a combination of potassium and sodium antimony tartrate, was used by the writer with success.

(*c*) Other antimony tartrate salts have been used, such as ammonium and lithium, but no particular advantage can be claimed for any of these.

(*d*) A pyrocatechin compound, which has been issued by the firm of von Heyden under the name Antimosan, has been used by the writer in the treatment of a number of kala-azar cases; it appears to have some advantage over the tartrates.

2. THE PENTAVALENT COMPOUNDS OF ANTIMONY

During the last few years a large number of these have been prepared and used in the treatment of kala-azar, and during the next few years it seems probable that their number will be added to to a considerable extent. A table showing the order in which these were introduced for the treatment of the disease is given below:

NATURE OF COMPOUND AS FAR AS KNOWN	SHORT CHEMICAL NAME AND TRADE NAME	INTRODUCERS AND MANUFACTURERS	REFERENCE TO FIRST CLINICAL REPORTS
Acetyl-para-amino-phenyl stibiate of sodium	Stibacetin 'Stibenyl'	Von Heyden, Dresden	(Italy) Caronia 1916 (England) Bahr 1920 (India) Mackie 1921 ,, Napier 1922*c*
Urea combined with para-amino-phenyl stibinic acid	Urea-stibamine 'Stiburea'	Brahmachari	Brahmachari 1922*a* Shortt and Sen 1923
Meta-chlor-para-acetyl amino-phenyl stibiate of sodium	'No. 471' 'Stibosan'	Von Heyden	Napier 1923*d*
Nitrogen-glucoside of sodium para-amino phenyl stibiate	Stibamine glucoside 'Neostam'	Wellcome Research. Burroughs Wellcome and Co.	Napier 1925*a*
Para-amino-phenyl stibinic acid combined with urea and glucose	'Aminostiburea'	Union Drug Co.	Napier 1925*c*
Para-amino-phenyl stibinic acid combined with an amine	'No. 693'	Von Heyden	Napier 1925-1926 and 1927

3. OTHER ANTIMONY PREPARATIONS

Antimony in a fine state of division, Brahmachari (1920), colloidal antimony, antimony oxide given intravenously, intramuscularly (Napier, 1923) and by the mouth (Rogers, 1919), Martindale's solution, i.e. antimony oxide in glycerine, and colloidal antimony sulphide (Rogers, 1919), are some of the preparations that have been tried.

A degree of success has been claimed for some of these, but the fact that the original introducers have apparently abandoned their use suggests that they did not prove to be as useful as was at first hoped; they need not, therefore, be considered in detail.

TOXICITY AND PHARMACOLOGICAL ACTION OF THE VARIOUS PREPARATIONS OF ANTIMONY

Fargher and Gray (1921) give the minimum lethal dose of sodium and potassium antimony tartrate respectively, as 25 and 18 milligrammes per kilogramme of body-weight. These doses were calculated by giving intravenous injections into the tail vein of mice. They give the minimum lethal dose of para-acetyl-amino-phenyl-stibiate of sodium (stibenyl) as 133 milligrammes. The toxicity of the first batch of stibamine glucoside was worked out at the Wellcome Research Institute; two out of ten mice died after a dose of 500 milligrammes per kilogramme of body weight.

Brahmachari (1922*a*) has worked out the relative toxicity of a number of antimony compounds, but he used guinea-pigs and gave the injections intramuscularly; by this method the results obtained are rather liable to be irregular, and it will be found more satisfactory to use the intravenous route for carrying out these comparative tests. The large variety of white mouse also has not been found to give very satisfactory results in the author's hands, and he prefers to use the small Japanese variety of mouse. This mouse usually weighs less than 10 grammes. The table below gives a summary of a number of toxicity experiments carried out during the last two years. The mice used were all of the Japanese variety and weighed less than 10 grammes.

TABLE

PREPARATION	Dose that at least 9 out of 10 mice survive ; mgm. per kilo	Dose that kills 3 out of 4 mice ; mgm. per kilo
Stibosan	200	275
Stibamine glucoside	300	500
Urea Stibamine and Stiburea	175	250
Aminostiburea	175	250
Von Heyden, No. 693	250	350
Sodium Antimony Tartrate	20	30

Chopra (1927) has studied the pharmacological action of a number of these compounds on cats. He found that they all had a depressing effect on the heart when injected into the blood stream. This was very marked in the case of Stibosan, less marked in the casê of urea-stibamine, and slight in the case of sodium antimony tartrate. There was a fall in the systemic blood pressure and a corresponding rise in the pulmonary blood pressure. There was an increase in the spleen volume ; this was very marked when any of the therapeutically active pentavalent compounds were injected, but less marked when sodium antimony tartrate was used.

It is rather interesting that, except for the fact that all the pentavalent compounds are tolerated clinically better than sodium antimony tartrate, there does not appear to be any relationship between the tolerated dose and the relative toxicity of the compound. No. 693, for example, is tolerated much better than stibamine glucoside, although it is more toxic to mice.

The depressing effect on the cat's heart was more marked when the clinically well-tolerated pentavalent compounds were administered than when sodium antimony tartrate was given. The marked increase in the volume of the spleen suggests that this may be one of the ways in which these compounds act. The largest number of parasites are always found in the spleen, and the more marked flushing effect after an injection of one of the pentavalent compounds may account for their greater efficiency in the treatment of the disease. The severe pain in the spleen, which sometimes occurs immediately after an injection, is also explained.

THE CHOICE OF ANTIMONY PREPARATION: TRI-VALENT OR PENTAVALENT ANTIMONY?

There are two antimony salts of tartaric acid which have come into general use, and there are a number of pentavalent compounds of proved value; the problem that now faces the practitioner is: Which of these shall I use? The preparations fall naturally into two groups, the tri-valent and the pentavalent, and the first point to be settled is which of these should be used.

The advantages which can be claimed for the pentavalent compounds are as follows:

(1) The compounds are much less toxic, and can, therefore, be administered in larger doses.

(2) The total amount of antimony which is necessary to effect a cure can thus be administered in a much smaller number of doses. About ten doses of the pentavalent compounds—against thirty doses of sodium antimony tartrate—can be looked upon as the average number necessary for an ordinary case. This reduction in the number of injections means a reduction in the time the patient is under treatment from two months to about three weeks, which, in its turn, means that a greater percentage of the dispensary class of patient will complete a full course of treatment.

(3) Resistant cases, which respond slowly when treated with the tartrates, frequently improve rapidly on the larger doses of antimony in the form of the less toxic pentavalent compounds.

(4) Certain disagreeable symptoms, such as coughing and severe joint pains, which are frequently associated with the tartrate treatment, do not occur when the pentavalent compounds are used. In a number of cases these symptoms are so severe that they necessitate the reduction of the dosage to such an extent as to prolong the course of treatment almost indefinitely and to reduce considerably the chances of an eventual cure.

(5) The death rate amongst kala-azar patients under treatment has been very markedly reduced since the introduction of the pentavalent compounds. One of the most frequent causes of death during the course of treatment, namely, pneumonia, has been almost entirely eliminated.

It is unfortunate that in reporting the results of treatment with any special form of antimony, writers usually omit all reference to the deaths that occurred; in a few instances in the literature this

very important piece of information has been given. Muir (1918) reported a 12 per cent. death rate in a series of 150 cases. Knowles (1920), in a series of 86 cases treated at Shillong with potassium antimony tartrate, reported a 25·6 per cent. death rate; this rate was exceptionally high because his wards were invaded by an epidemic of influenza and a number of his patients that were almost ready for discharge died. The present writer (1924*b*) reported 14·4 per cent. deaths in a series of 139 cases treated in 1922 with sodium and potassium antimony tartrate, but in a smaller series of 35 cases, treated with sodium antimony tartrate, the death rate was 22·8 per cent. (Napier, 1926).

The foregoing figures are in marked contrast to the 4·2 per cent. deaths in a series of 167 cases treated in the Carmichael Hospital for Tropical Diseases during 1925, with six different pentavalent compounds (Napier, 1926).

Each of the advantages enumerated above contributes to a general increase in the cure rate.

The disadvantages of the pentavalent compounds are few. It seems possible that post-treatment jaundice is a little more frequently encountered in patients who have received one of these compounds, and with some of them an anaphylactic-like group of symptoms appears suddenly towards the end of the course of injections, but neither of these complications can be considered as a serious one.

It is thus obvious that there are practically no special advantages in using the antimony tartrate salts, and there is only one thing that stands in the way of the total abandonment of these in favour of one or other of the pentavalent compounds; this is the relatively very high cost of the latter.

This high price is not so serious a matter to the patient who can afford to be treated by a private practitioner, as he is able to effect a saving on the number of attendances necessary from this doctor, but it is a very serious matter for the authorities responsible for the treatment in hospitals and charitable dispensaries. During the last year, since a number of separate commercial interests have been competing for the same market, the prices of the various pentavalent compounds at present on the market have dropped at least 50 per cent., and a further drop is likely to occur, but, as the process of manufacture of these compounds is an expensive one, the cost will

never be comparable to that of sodium antimony tartrate. Taking three grammes as the dose necessary to effect a cure, the cost of curing one patient will be three pence if sodium antimony tartrate be used, and at least 25 shillings if one of the pentavalent compounds be used.

It is obvious that, despite the many advantages of the pentavalent compounds, sodium or potassium antimony tartrate will still have to be used to treat the greater bulk of the many hundreds of thousands of patients suffering from kala-azar in Assam, Bengal and in China; the treatment with these salts will, therefore, be described in some detail.

Other Drugs That Have Been Tried

The other drugs that have been tried in the treatment of this disease are too numerous to record. None of them has apparently had the least effect on the course of the disease. Soamin often causes some improvement in the general condition of the patient and has been given in conjunction with antimony tartrate in a number of cases with success, but no specific properties can be claimed for this compound. Salvarsan has proved a complete failure. One of the latest fashions in treatment, intravenous injection of tincture of iodine, has been tried with entirely unfavourable results.

The writer (1923*c*) gave a course of injections of Bayer 205 in a short series of cases, and concluded that this drug had no therapeutic effect in the disease. He also gave two patients a number of injections of Tryparsamide; both these patients became so ill that this treatment had to be abandoned in favour of sodium antimony tartrate, to which they soon responded.

Treatment by the Antimony Tartrates

Except in the presence of definite contra-indications—see below—the antimony injections should be commenced in every instance immediately a definite diagnosis is arrived at. If the patient is very debilitated the injections should be given cautiously in minimal doses.

CHOICE OF SALT

It is not a matter of very great importance which salt is used, but it is of great importance that the salt should be of a good

quality. Fortunately, there is little trouble about this nowadays, as most drug manufacturers are making these salts especially for intravenous injection. It is claimed by some manufacturers that the scale preparation of sodium antimony tartrate is the purest. It dissolves rapidly, leaving no deposit; this cannot be said of many other samples. Apparently there is some difficulty about preparing the potassium salt in scale form, but a triple salt, potassium sodium antimony tartrate, in scale form, is available and has given good results.

STRENGTH, PREPARATION, AND PRESERVATION OF SOLUTIONS

The strength of the solution is again a matter of no very great importance. One per cent. and two per cent. solutions are the strengths most frequently used, but more concentrated solutions can be used. The writer used a five per cent. solution in his wards for some time, but it was found that, although nine out of ten patients tolerated this strength, even with a small dose coughing and retching sometimes occurred in a patient who could tolerate relatively much larger doses of a two per cent. solution. As it is obviously dangerous to have solutions of two different strengths in use at the same time, a return to the two per cent. solution was made.

A two per cent. solution will be found the most satisfactory for routine purposes.

It is very important that freshly prepared solutions should be used, as moulds are liable to grow in the solution, splitting the tartrate molecule and precipitating the antimony in the form of oxide. The oxide itself is not toxic, but the solution that is left is occasionally very toxic; it may give rise to rigors, and even death has been reported after the administration of such solutions.

Solutions may, however, be kept for quite a considerable time—for more than a month—if 0·25 per cent. of carbolic acid be added at the time of preparation. It is far more satisfactory to make a stock solution, say about once a fortnight, than to make a fresh solution each day; in the latter case it probably has to be made hurriedly on a busy day, whereas in the former it can be made leisurely at any time on any day of the week.

The solutions should be made in distilled water, if possible, but ordinary filtered tap water can be used without ill effects, either

with or without the addition of 0·85 per cent. sodium chloride. The solution when prepared should be brought to the boiling-point and allowed to boil gently for a minute or two, but not sufficiently long to cause any appreciable evaporation. It is not advisable to 'autoclave' the solutions, as at high temperatures decomposition is said to occur.

The solutions should be prepared and kept in flasks. These can be plugged with cotton-wool whilst being stored. When they are required for use the cotton plug is removed, the mouth of the flask flamed, and a rubber cap placed over it. The rubber caps should be kept in a wide-necked bottle containing alcohol, removed by a pair of forceps, allowed to dry in the air, washed with a small amount of sterile distilled water, or of the solution itself, to remove the last trace of alcohol, and then drawn over the mouth of the flask. Immediately before use the cap of the flask should be swabbed with spirit, and the syringe can then be filled by puncturing the cap of the inverted flask.

When only small doses are to be given, small soluble tablets, which are now obtainable, can be dissolved in the necessary amount of sterile distilled water immediately prior to use.

DOSAGE

The dosage will naturally vary according to the circumstances. Assuming that a two per cent. solution is used, for the average adult patient the first dose should be 2 cc. (i.e. 0·04 gramme of the salt); doses should be increased on each occasion by 1 cc. up to a maximum of 5 cc. (i.e. 0·1 gramme); subsequently 5 cc. should be given on each occasion. For more debilitated patients 1 cc. can be given as an initial dose, and the doses increased by 0·5 cc. up to a maximum of 5 cc.

For infants of three years it is advisable to commence with 0·5 cc. and to make 2 cc. the maximum. For children of twelve years the maximum should be 3·5 cc. The doses for the intermediate ages are in proportion. For a very debilitated patient it may be advisable to make the maximum about 3·5 cc., as in the case of children. It will be noticed that though one is guided to a certain extent by the age of the patient, proportionately larger doses are given to children.

The injections should be given on alternate days throughout the

course of treatment. It is permissible to extend the interval by one day in certain cases. On no account should the injections be given less often than twice a week, and the best results are obtained if they are given on alternate days.

TECHNIQUE OF INTRAVENOUS INJECTION.

The technique for veni-puncture has already been given (page 104). The procedure described is followed up to the point of puncturing the vein, except that the syringe should previously be loaded with the dose that is to be given. Directly the vein is punctured blood will rush into the barrel of the syringe ; the congesting band is now released and the solution injected slowly. Some writers lay special stress on the importance of injecting very slowly. The present writer has seldom found it necessary to pay any special attention to this point. In a few instances coughing has been caused by too rapid injection, but most of the patients who cough will do so whether the injection be given rapidly or slowly.

It is very necessary that during the loosening of the constriction and during the pressing home of the piston the person injecting the solution should not to the least extent alter his grip on the barrel of the syringe.

He should also see that the distal phalanx of his little finger is resting on the arm of the patient so as to steady his hands. Otherwise the point of the needle may shift a little and be withdrawn from the vein or pierce the opposite coat of the vein. If during the pressing home of the piston the operator at any point becomes doubtful as to whether the point of the needle is still within the lumen of the vein, he can satisfy himself by slightly withdrawing the piston. If the needle is in position blood will again flow into the syringe. Care must be taken that no air escapes into the vein from the syringe.[1] This will be most apt to occur when the piston does not fit tightly in the barrel or where the needle does not fit tightly on to the syringe.

[1] A considerable amount of difference of opinion exists on the subject of the danger of giving air into the veins. The fear of injecting air should never detur anyone from giving intravenous injections, but, as the introduction of air cannot possibly do any good, it is better to avoid injecting it. The writer, who can claim an experience of over 10,000 intravenous injections, admits that there is probably no danger in giving one cc. of air into the veins, but prefers neither to risk the experiment nor to encourage others to risk it.

The subcutaneous injection of most of the salts used for intravenous injection will cause a very painful swelling of the arm and often abscess formation, but there is no excuse whatsoever for the occurrence of this accident. The writer's assistant can claim to have given 30,000 consecutive injections without causing any painful swelling of the arm. It may be necessary to make half a dozen attempts to puncture a vein, or, in extreme cases, to give up the attempt altogether for that day, but solution must never be injected until one is absolutely certain that the point of the needle is in the lumen of the vein. It is well to note, in passing, that the art of giving intravenous injections is worth acquiring, as it is becoming more and more necessary for the successful treatment of diseases other than kala-azar.

THE COURSE OF THE DISEASE UNDER TREATMENT

The Fever.—The patient should be warned that the fever is not likely to subside before about ten injections have been given, and that if it does so there is some likelihood of its reappearance within a short time. The average time before the disappearance of the pyrexia is about four weeks or after twelve injections. There are some cases in which the temperature falls after about the third injection and does not rise again, but there are also a few cases in which the fever does not subside for a considerably longer time than a month. It is not a signal for despair even if the temperature remains above normal after as many as 40 injections. A few cases show a slight reactionary rise of temperature after each injection. In a case running an afebrile or almost afebrile course the commencement of injections of antimony tartrate frequently causes the reappearance of a remittent or intermittent type of pyrexia.

General Condition.—In the majority of cases the patient improves in appearance within a very short time of the commencement of treatment. The hair ceases to fall out and commences to regain its natural lustre; the appetite, if it was ever bad, increases and the digestion is improved, and, although the patient may be running a comparatively high temperature, he will tell you that he feels considerably better. There is a certain amount of danger of the appetite improving in advance of the digestion, with the disastrous consequence that the patient may seriously over-eat and thereby acquire an intractable diarrhœa. Most patients do not put on weight

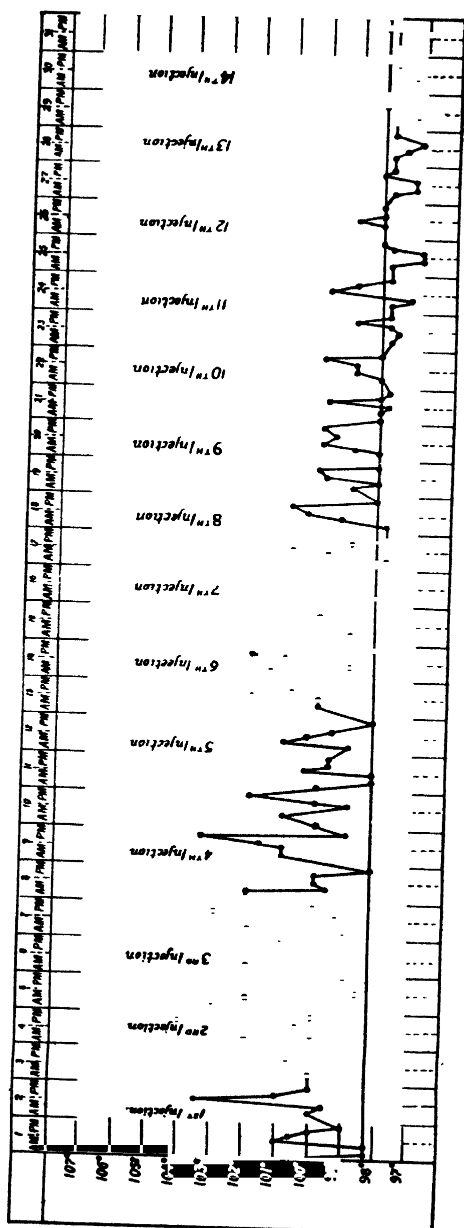


CHART IX

Four-hourly chart showing the typical course of the disease in a case treated with sodium antimony tartrate.

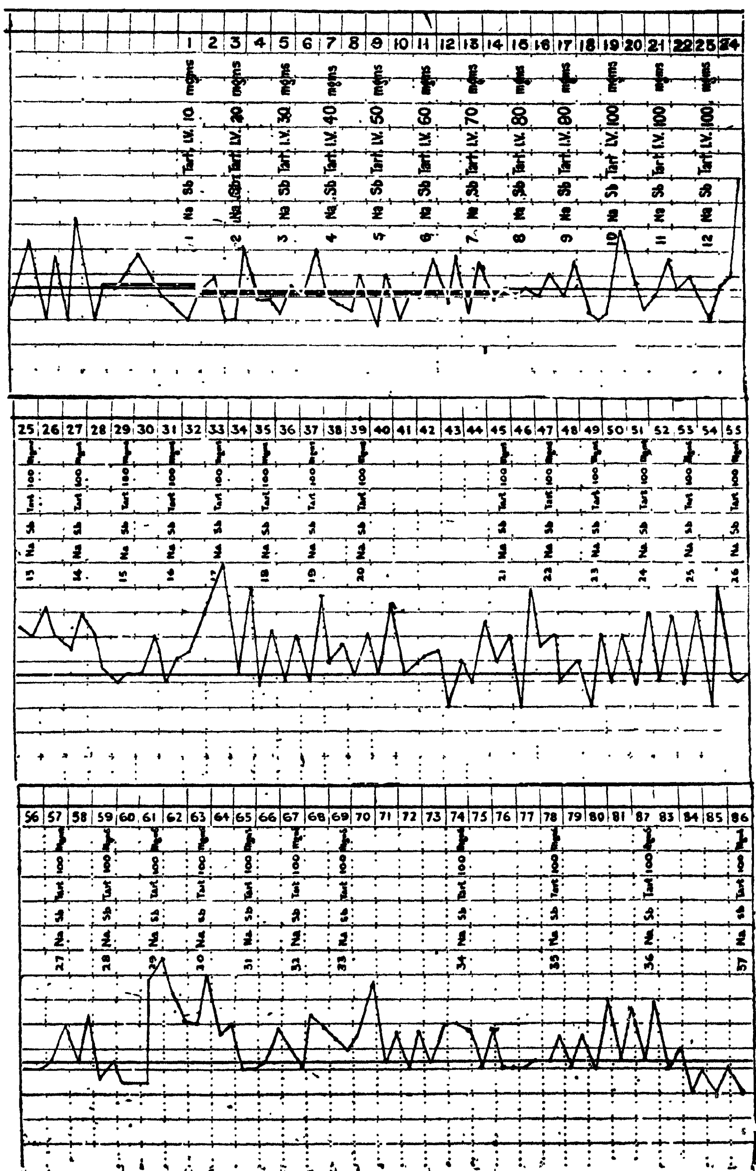


CHART X

Morning and evening temperature record in a case in which there was delayed reaction to treatment with sodium antimony tartrate.

from the commencement of treatment; it is far more frequently the case that the patient will actually lose weight for the first few weeks. It is a rather remarkable fact that many patients who have otherwise made an uninterrupted recovery have not put on weight until the last week or so of treatment. This loss of weight, or failure to increase in weight, is often due to the disappearance of œdema and decrease in the size of the spleen. On the other hand, there are a few patients who commence to put on weight immediately and continue to do so throughout the course of the treatment. See Chart XII.

In women the menses do not usually recommence until after the course of treatment has been completed.

The Spleen and Liver.—The behaviour of the spleen is by no means consistent; in some cases the spleen diminishes rapidly in size from the beginning and disappears under the costal arch before the patient goes out of hospital. We have felt spleens reaching to within an inch of the symphysis pubis which have disappeared under the costal arch within three months. On the other hand, in some cases there is very little decrease in the size of the organ. The behaviour of the spleen is naturally dependent on its consistence. In a long standing case, in which the spleen is very hard and fibrous, diminution in size beyond a certain point is naturally impossible, but a spleen that has grown rapidly will as rapidly decrease. Furthermore, if kala-azar has supervened in a chronic malarial subject who previously had an enlarged spleen, one cannot expect that organ to subside completely after anti-kala-azar treatment.

Generally speaking, the diminution in size of the liver is less noticeable than that of the spleen.

The Blood.—The blood picture improves rapidly under treatment. By the time the patient has completed his course the white blood count has returned to normal, and it is by no means infrequent to find a distinct leucocytosis. In the differential count the most noticeable increase is in the eosinophiles. The red blood count should also be nearing the normal point.

Length of the Course of Treatment—The length of time for which the treatment should be continued is an ever-ripe subject of discussion. It is as difficult to lay down any fixed rule on this subject as it is necessary that the general practitioner should have some fixed rule on which to work. As some patients appear to be

absolutely resistant to treatment and others show varying degrees of resistance, it is obviously not possible to frame a rule to fit every case. Treatment should certainly not be discontinued when the acute symptoms subside, nor, on the other hand, is it necessary to wait until the patient has returned to his original state of health, that is to say, until his spleen is no longer palpable and his weight is again normal.

The size of the spleen will continue to diminish and the general condition of the patient will continue to improve after the specific treatment has been discontinued.

The aldehyde reaction of the serum of a patient who has just completed his course of treatment and is cured is very often still quite strongly 'positive,' so that this test is of no value in deciding whether or not a patient is cured. It will, however, be noted that the aldehyde reaction will in almost every cured case be quite 'negative' six months after the patient has completed the full course.

Knowles (1920) suggested that for an adult a total of two grammes was a sufficient course to ensure cure, but he was working in a favourable climate and, furthermore, he has subsequently heard that some of his cases, patients whom he had considered cured, have now relapsed. Muir (1918) stated that a four months' course of thrice weekly injections should be given in every case, but did not lay emphasis on the total amount of the drug that was necessary. It will be seen that, following the course he suggested, a total of 5 grammes would be given in the time under ordinary circumstances.

The routine that was adopted in the Carmichael Hospital for Tropical Diseases was to give a full course of 30 injections to each patient. A spleen or liver puncture was then done, and two or three N.N.N. tubes were inoculated with the material obtained. If no flagellates developed in the N.N.N. tubes and the patient's general condition and blood count were satisfactory, he was discharged as cured; otherwise he was given a further course of ten to fifteen injections and the spleen puncture repeated.

It is obvious that it is not possible for the general practitioner to adopt the above method to ascertain whether the patient is cured or not; nor is it advisable, or even possible, for him to give every patient, as has been suggested, a full course of 5 grammes of the salt. The following rules for the length of the course of treatment will be found useful:

(1) Some cases are definitely resistant to treatment with antimony tartrates, and should therefore not be provided for in ordinary rules for treatment.

(2) The maximum total dose of sodium or potassium antimony tartrate that is necessary to effect a cure, in any but a definitely 'resistant' case, is 4 grammes per 100 lbs. body-weight of the patient.

(3) This maximum course of treatment can, under certain circumstances, be modified; if the author's rules for dosage have been adopted, and provided there has been a decided increase in weight, the spleen is reduced to the level of the costal arch, or, in cases where it was very markedly enlarged, by at least four inches, and the white blood count is above 6,000 per c.mm., then in cases whose temperature comes to normal by the seventh injection, 30 injections (i.e. 2·88 grammes in an adult) should be given; in cases whose temperature falls to normal after the seventh but before the tenth injection, 35 injections should be given; in cases whose temperature falls to normal after the tenth but before the sixteenth injection, 40 injections should be given; and those cases whose temperature continues for longer than this should be given a full course of injections, bringing the total dose up to 4 grammes per 100 lbs. weight of the patient.

If after this course of treatment the condition is still not cured or relapses after a short interval, it is obvious that the case can be classed as a 'resistant' one, and that treatment with one of the more powerful pentavalent compounds should be undertaken.

Interruptions of Treatment on Account of Complications.—It is of the utmost importance that when the treatment is once begun it should be continued uninterruptedly. It may be necessary to discontinue the injections of antimony tartrate when symptoms appear which are directly due to the effects of the salt that is being given, but when the common complications of the disease itself arise it is seldom advisable to do so. It has been found that slight diarrhœa, œdema of the feet, the presence of a trace of albumen in the urine, severe bleeding from the gums, are indications that the antimony tartrate treatment should be commenced immediately, or, in cases where it has already been commenced, should be continued. But in view of the fact that there is now an alternative form of treatment available, it is doubtful if one

is justified in attempting to treat the patient with lung complications by these salts. In cases where there is severe dysentery it is as well to treat this condition first, and to postpone the specific treatment until the condition of the patient has improved.

The tendency is to discontinue the antimony tartrate injections too readily, but, as continuity of treatment is an extremely important point, this tendency should be combated as far as possible.

COMPLICATIONS DUE TO ANTIMONY TARTRATE INJECTIONS

There are certain complications arising which are due to the antimony injections.

Coughing.—The commonest of these is severe coughing immediately after the injection, which may be so severe that the patient eventually vomits. It is an extremely difficult complication to circumvent. It is always an indication that the dose should not be increased. By decreasing the dose and then very gradually increasing it again a certain degree of tolerance will be acquired. In some instances this symptom may become so persistent that it will be necessary to abandon this form of treatment.

Vomiting is usually an indication that there is something wrong with the solution that is being given, but it also occurs in some cases where the solution is perfectly satisfactory. Both vomiting and coughing may be induced by giving the injections on a full stomach or by injecting the solution too rapidly. Codein grs. ¼ or liquor adrenalin min. X given intramuscularly about 20 minutes before the injection of the antimony salt will often reduce the tendency to coughing.

Pneumonia and Lung Complications.—The extreme frequency of the occurrence of respiratory complications during the course of treatment with the antimony tartrates, led one to suspect that the condition was actually caused by these injections; and the extreme rarity of these complications amongst patients treated with the pentavalent compounds has now confirmed this suspicion.

Pneumonia is one of the most distressing complications, as it so frequently carries off a patient who has completed about half his course of treatment and is progressing favourably.

Kidney and Bowel.—The kidney and bowel complications are probably not caused, but are very possibly aggravated, by the antimony tartrate injections.

Joint Pains.—Severe joint and muscular pains are very common complications, but, fortunately, seldom occur except towards the end of a course of treatment. They usually come on some four or five hours after the injection, and last for anything up to twelve hours. We have found that by giving 20 grains of aspirin about half an hour before the pains are expected to commence, the severity of these can be diminished considerably.

A less common complication is an acute arthritis, particularly effecting the wrist joints, the knee joints and the ankle joints. This usually subsides in about ten days, and the general condition of the patient is much improved by this attack.

Eruptions.—An irritating papular eruption occasionally occurs on any part of the body in cases under treatment, which is possibly caused by the antimony tartrate injections. This does not usually disappear until the injections have been discontinued.

The Heart.—Very marked slowing of the heart is met with in some cases towards the end of the course of treatment, and is certainly an indication that the treatment should be suspended.

Other Symptoms.—A very sharp reactionary rise of temperature occurs in a small percentage of cases, and in a few rigors have occurred, but these again are usually due to some abnormality in the solution injected. In one case very profuse sweating always occurred immediately after the injection, and in another case the injections were followed by fainting fits. During the administration of therapeutic doses the fall of blood-pressure is very small and only transitory. It is scarcely appreciable with the ordinary clinical manometer. Severe headaches occasionally follow the injections. A case is reported of a patient who stopped breathing after an overdose of antimony tartrate, and was revived after 20 minutes' artificial respiration. A metallic taste in the throat is often noticeable after an injection, but is a symptom of no particular significance.

Treatment by the Pentavalent Antimony Compounds

The treatment should be commenced immediately a diagnosis is made. If the patient has obviously been neglected or starved, a few days in bed on a generous diet before the injections are commenced may be advisable. In early cases—the only cases in which there is likely to be any doubt about the diagnosis—there is little

harm in delaying the specific treatment for a few days, or even a couple of weeks, whilst the diagnosis is being confirmed, but once the diagnosis is made the injections should be commenced. If the disease is in an advanced stage and the patient is very debilitated the injections should be given cautiously, but as the debility is likely to be progressive there is nothing to be gained by delay.

CHOICE OF COMPOUNDS

Although it is quite obvious that many of the pentavalent compounds are infinitely more valuable than the antimony tartrates, it is difficult to decide which of these compounds at present available is the most valuable in the treatment of kala-azar. A number of points have to be considered, namely, the relative inocuity of the compound and the death rate amongst patients treated with it, the total amount necessary to effect a cure and the maximum tolerated individual dose, the complications and sequelæ associated with the use of the particular compound, its stability, and its cost of manufacture.

As each individual is not the same, and a total dose which will cure one patient will not cure another, the question of total dosage should be discussed in terms of percentage cure rate for each total dose, and, as the usual rules of dosage probably also apply to these compounds, the dose taken into consideration should be the dose relative to the body-weight of the patient concerned. A 100 per cent. cure rate is at present beyond attainment, so, for the purpose of comparing different compounds, some easily attainable rate, such as 90 per cent., should be chosen.

The importance of taking into consideration the maximum tolerated individual dose is obvious, as the number of injections necessary to effect a cure is dependent on this factor also. The actual amount of sodium antimony tartrate and Stibosan that will produce a 90 per cent. cure rate amongst those that survive the course of treatment is probably about the same in each instance, but, as the maximum tolerated dose of the latter is three times as great as that of the former, a course of treatment with the former compound takes just three times as long to complete as does the treatment with the latter.

The question of the stability of the compound comes in when the treatment of large numbers of patients has to be considered.

Many of the compounds in use have to be kept in sealed ampoules, as they are very hygroscopic and undergo a chemical change when kept in contact with air. It is obvious that compounds which can be stored in ordinary stoppered bottles and weighed out when required are the most useful for general purposes.

Meta-chlor-para-acetyl-amino-phenyl stibiate of sodium — von Heyden No. 471—Stibosan.—This compound is in the form of a light brown powder, it is non-hygroscopic, and does not undergo any change when kept for a considerable period (two years) in an ill-corked bottle at ordinary room temperature in Calcutta. It is obtainable in sealed ampoules or in stoppered bottles. It is easily soluble in distilled water, and forms a sterile solution which does not apparently undergo any rapid change.

The author (1926*b*) published a paper giving the details of the treatment of the first 104 cases (including the 11 cases reported in 1923) treated with this compound. The death rate of this series was 10·6 per cent.; of the survivors, two did not respond to treatment, both these cases proved absolutely resistant to all forms of antimony, and eventually died. The cases in this series were patients who had either had no previous treatment or had relapsed after a definite course of treatment. Of the patients that were discharged cured, 77 were traced for six months after their discharge; of these, 70 remained well and 7 relapsed.

The mean total dose of this series was 2·78 grammes.

The mean number of injections given to each patient was 13·3. Calculated with reference to the body-weight of the patient, the dose per 100 lbs. body-weight of patient was 4·00 ± ·97 grammes.

Of the previously untreated patients who received a relative dose (i.e. dose per 100 lbs. of body-weight)—

of between 2 and 3 grammes ..	18·2 per cent. relapsed.
of those receiving between 3 and 4 grammes	9·2 per cent. relapsed.
of those receiving between 4 and 5 grammes	5·0 per cent. relapsed.
and of those receiving more than 5 grammes	none relapsed.

The injections were given three times weekly, and 0·3 gramme was the maximum dose.

Greig and Kundu (1925), in a much smaller series of cases, gave a mean dose of 2·0 grammes, and found the mean of the doses per 100 lbs. weight of patient necessary to effect a cure to be 2·73 grammes; they claimed that all these cases were cured, but did not follow them up.

Para-amino-phenyl-stibinic acid combined with urea — Urea-stibamine—Stiburea.—This compound is in the form of a light brown powder. It undergoes a chemical change if kept in contact with the air for any time; it is, therefore, supplied only in sealed ampoules. It is easily soluble, and in sterile distilled water it forms a sterile solution.

There have been many reports published on cases treated with this preparation, but most of the reports are unsatisfactory in that they are based on a very small number of cases; these are often collected cases, and not a consecutive series. Greig and Kundu (1925) report the results of treatment of 51 patients; they do not claim that they are consecutively treated cases. They do not report any deaths, but they do not claim that there were none, and they refer to a very resistant case in which 10 grammes of the preparation were given without producing any improvement, but do not include this case in the series. They relied entirely on spleen or liver punctures, with culture of the material, as proof of cure, and did not follow up the cases. Some of their cases had received previous treatment. They gave an average dose of 2·12 grammes, which was equivalent to 2·4 grammes per 100 lbs. weight of patient. They gave 0·25 gramme as a maximum dose, and gave the injections on alternate days.

Another series was reported by Foster (1924) and Banerjee (1925). The patients in this series were members of a tea garden coolie force; they were for the most part previously untreated. Proof of cure was by spleen puncture only. There were 4 deaths in a series of 67; the death-rate was thus 6 per cent. The mean dose of the series was just about 2·00 grammes, but more than half the patients were children, and the relative dose was not worked out. The mean number of injections given to each patient was about ten.

The present writer (1924) treated a few cases in the early days of urea-stibamine, but had poor results. His subsequent experience suggests that he was unfortunate in receiving samples from an unsatisfactory batch. During the last year he has treated 48

patients, with only three deaths. The mean total dose of this series was 2·08 grammes, the relative dose 2·7 grammes, and the mean of the number of injections given in each case was 11·8. Only one relapse has so far been reported; a spleen or liver puncture with culture was done on each of these cases before discharge, and 36 of them are known to have remained free from any signs of the disease for at least six months.

Stibamine glucoside—Neostam.—On the treatment with this compound so far few reports have been published. The writer (1925*a*) reported on ten cases. There was one death in this series. Nine patients were discharged cured on the strength of a negative liver puncture culture; of these, seven are known to have remained free from the disease since. The mean total dose was 2·58 grammes, or 4·21 grammes per 100 lbs. body-weight of patient, and the mean number of injections was 13·8. It seems possible that an unnecessarily high total dose was given in these cases, as Greig and Kundu (1925) subsequently cured two patients with doses of 1·85 grammes and 2·05 grammes respectively.

Para-amino-phenyl-stibinic acid combined with urea and glucose—Aminostiburea.—This is a comparatively recently introduced compound; the addition of glucose is reputed to add both to the stability and to the diffusibility of the drug.

The writer has now treated 52 patients with this compound. There were two deaths in this series, one patient developing pneumococcal meningitis and dying within 36 hours of the onset of symptoms, and two very resistant cases which did not respond to treatment. The mean total dose of the series was 2·4 grammes, which is equivalent to a dose of 3·35 grammes per 100 lbs. weight of patient, and the mean of the number of injections given to each patient was 12·06. All the patients were discharged after a negative spleen or liver puncture culture, no relapses have been reported, and 35 of them are known to have remained well up to six months after discharge.

Para-amino-phenyl-stibinic acid combined with an amine—von Heyden 693.—The present writer (1925, 1926 and 1927) has been using this compound for some time, but it is not yet obtainable for general use. A series of 61 patients have now been treated without any deaths; 50 of these have remained well for periods of not less than six months. The mean total dose was 2·19

grammes, which represents 3·35 grammes per 100 lbs. body-weight of patient, and the mean total number of injections given to each patient was 10·85. Two patients showed no improvement; both of these were resistant to all forms of treatment, and died about one year after discharge. No relapses have been reported.

Discussion.—It is obvious that the reports on the various compounds are not strictly comparable. Greig and Kundu consider the mean 'sterilizing dose' per kilogramme of patient to be 0·054 grammes and 0·06 grammes respectively, for urea-stibamine and Stibosan; they conclude that there is little difference in the curative value between these two compounds. The present writer, on the other hand, gave a mean relative dose of 4·01 grammes of Stibosan and obtained a nine per cent. relapse rate, whereas by giving a relative dose of 2·7 grammes of urea-stibamine he obtained a lower relapse rate. In the Aminostiburea series there were a number of resistant cases, patients who had undergone a course of injections with both urea-stibamine and Stibosan without being cured; these were given a somewhat extended course of treatment, but were eventually cured.

The number of injections administered was about the same in each series, but it was lowest in the No. 693 series.

The time of cessation of fever appears to give some indication of the rate of progress of the patient, and therefore of the relative efficacy of the compound used. The mean number of injections prior to the cessation of fever of each series was as follows: Stibosan, 5·6; Stibamine glucoside, 5·4; Urea-stibamine, 5·1; No. 693, 4·57; and Aminostiburea, 4·48.

Stibosan shows the highest death rate and No. 693 the lowest; in view of the fact that the cases were not in any way selected, and that a number of very debilitated patients were included in the series, it is rather remarkable to be able to report a series of 61 cases without a single death.

One of the common complications of treatment with Aminostiburea and urea-stibamine, a condition suggesting anaphylaxis, which will be referred to later, is rare with Stibosan, and has not been observed in any of the cases of the No. 693 series. Jaundice, which is a common sequel to the treatment with most of the pentavalent compounds, also appears to be rare with No. 693.

In conclusion, one may say that Stibosan is the most stable compound, that Aminostiburea is possibly the most powerful, but

that No. 693 is certainly the most innocuous, and, as its curative value, if less, is only slightly less than that of Aminostiburea, it will produce a cure in the shortest period, and should prove the most useful drug in the treatment of kala-azar.

PREPARATION OF THE SOLUTION

Most of the compounds are supplied in sealed ampoules containing the exact amount that is required for the dose. If this is the case, the top of the ampoule should be broken off and the contents poured into a test tube containing a convenient amount of sterile distilled water; not less than 3 cubic centimetres for 0·2 grammes of the compound. All the compounds referred to above are easily soluble, making solutions varying in colour from a light straw colour to a rich brown. The syringe can then be filled and the injection made in the usual way. If the syringe has been sterilised in alcohol, it is essential that all the alcohol should be washed out of the syringe before it is loaded.

If the compound is supplied in a stoppered bottle, the necessary amount of the compound should be weighed out on a piece of clean white paper on an accurate balance, and added to sterile distilled water as above. When a number of patients are to receive injections it will be found convenient to use a five per cent. solution; 2 cubic centimetres of a solution of this strength is equal to 0·1 gramme of dry powder.

Whenever possible, the solution should be prepared at the time the injection is to be given, but it is always possible to keep solutions for a few hours, and the writer has frequently given the solution of Stibosan 48 hours after it had been prepared without any ill effects.

DOSAGE

The initial dose of urea-stibamine (or Stiburea), Aminostiburea, and Neostam that is advocated by the manufacturers is 0·05 gramme, and they suggest increasing the dose by 0·05 gramme at each injection up to 0·2 gramme. The writer, however, gives 0·1 gramme as an initial dose, 0·2 gramme as a second dose, and 0·25 gramme for each subsequent dose. With Stibosan and No. 693 an initial dose of 0·2 gramme and subsequent doses of 0·3 gramme can be given. Children should be given smaller doses, but they

will tolerate proportionately much larger doses than adults. Infants from eighteen months to three years will tolerate a dose of 0·1 gramme, children of six years 0·15 gramme, children of ten years 0·2 gramme, and children of fourteen years 0·25 gramme. In treating debilitated patients it is advisable to start with a small dose and increase the dose very cautiously.

CHART XI

Four-hourly chart showing characteristic rapid reaction to treatment when one of the pentavalent compounds of antimony is used. The doses administered were 0·1 gramme, 0·2 gramme, 0·3 gramme and subsequently 0·3 gramme; altogether ten injections were given. There was a slight febrile reaction after the fifth and sixth injections but subsequently the temperature remained absolutely normal.

THE COURSE OF THE DISEASE UNDER TREATMENT

The Fever—After about the fifth injection the patient is usually entirely free from fever; there are, of course, many cases in which the fever disappears after the first or the second injection, and in most cases a downward tendency on the temperature chart will be noted immediately, but there are a few cases in which a temperature up to about 100° F. persists throughout the course of injections, only falling down to normal when the treatment is discontinued.





PLATE XVII

A child suffering from kala-azar: before treatment, and two months later, five weeks after treatment had been completed.

A reaction rise after each injection will be observed in a few instances, but a sudden sharp reaction in a patient who has previously not shown any reaction rises usually indicates that too big a dose has been given.

General Condition.—The general condition of the patient improves from the commencement of the treatment and it is the rule

CHART XII

Four-hourly temperature record of a case in which there was a rapid reaction to treatment with a pentavalent compound of antimony. The dosage was the same as in the previous case, Chart XI.

for the patient to announce that he feels much better some days before his temperature has returned to normal.

Weight.—As with the antimony tartrate treatment, the weight of the patient tends to decrease at first, after which it increases very rapidly: see Chart XIII.

The Spleen.—The spleen usually decreases very rapidly, and in the majority of instances it will have disappeared below the costal margin by the conclusion of the treatment; in some cases the decrease is slower, and in a few only a very slight decrease will be appreciable, but in these instances the spleen usually continues to decrease for some time after the treatment has been completed.

The liver, on the other hand, seldom shows much tendency to decrease until the injections have been discontinued.

LENGTH OF THE COURSE OF TREATMENT

Here one is faced with the same problem as in the case of the tartrate treatment. A spleen or liver puncture gives the best immediate indication of cure, but this is by no means an infallible test. The process of cure apparently continues for some time after the last injection has been given, and it has frequently been found that a patient who gives a 'positive' spleen or liver puncture a few

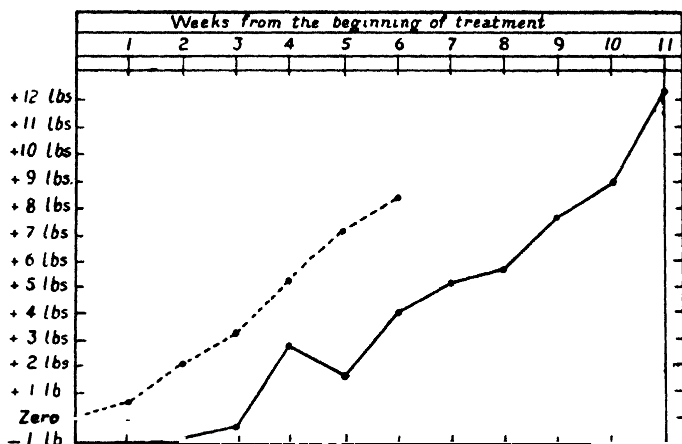


CHART XIII

Weight chart showing increase of weight during treatment.

The continuous line shows the average weights of 20 adult patients under treatment with sodium antimony tartrate. The dotted line shows the average weights of 20 adult patients under treatment with a pentavalent compound of antimony (von Heyden 693); there was an initial drop in weight in seven of these cases but this is overshadowed by the marked increase in the other cases.

days after the last injection will never show any further symptoms of the disease and that another puncture done some weeks later will be 'negative.'

No dose, however large, can be guaranteed to produce a 100 per cent. cure rate.

The best method to adopt is to give a good sound course of treatment and then, if a relapse occurs, give another much more severe course. For Stibosan the writer (1926*b*) suggested that a course amounting to 3·5 grammes per 100 lbs. weight should be given to each patient, and that with this course a nine per cent. relapse rate could be expected. The doctor will be influenced by the circumstances, but from the point of view of economy of material

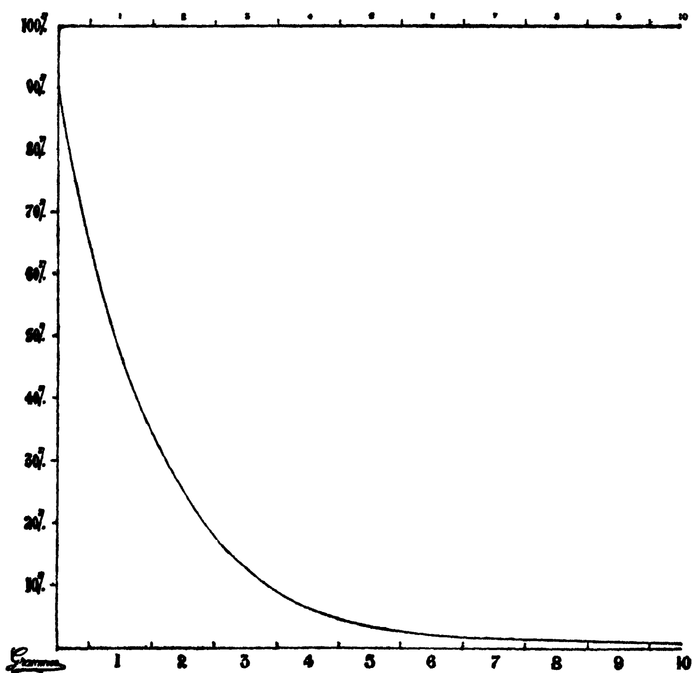


CHART XIV

A rough indication of the relapse rate that may be expected after various doses of Stibosan. The doses are calculated for an adult weighing 100 lbs.

when a large number of patients have to be treated the dosage suggested above is the best. An average adult Indian, weighing 90 lbs., would thus require a course of about eleven injections.

Experience suggests that in the case of some of the other pentavalent compounds a total dose of less than this amount will produce as great a cure rate.

In the urea-stibamine series the mean relative dose was 2·7 grammes. Very few patients received an actual dose of more than 2·5 grammes and the relapse rate was very low. Few relapses need be feared if a relative dose of 3 grammes be given; this means that if 0·2 gramme is made the maximum dose the majority of the patients will require between twelve and fifteen injections.

Although in the opinion of the writer Aminostiburea is a slightly more powerful antimonial, the same rules of dosage can be applied to this compound.

The mean total dose in the No. 693 series was 2·19 and the relative dose was 3·35 grammes; no relapses have occurred in this series. The routine adopted with this compound has been a course of 10 injections, amounting to a total of 2·7 grammes during a period of 22 days. But, as most patients over 70 lbs. will tolerate the maximum dose of 0·3 gramme, it will probably be possible to cut down the number of injections to eight in many instances without allowing the total relative dose to fall below 3 grammes; with this dose a very high cure rate can be expected.

COMPLICATIONS ASSOCIATED WITH TREATMENT BY THE PENTAVALENT COMPOUNDS

Lung complications are exceedingly rare and coughing never occurs as a direct result of an injection.

Vomiting.—This is one of the commonest complications. With Stibosan it is rare, except when the injections are pushed beyond the usual maximum dose, but in one case, an adult, vomiting was persistent whenever a dose of more than 0·15 gramme was given; with urea-stibamine, Aminostiburea and No. 693 vomiting occurs in about one in every ten cases, and in these the dose has usually to be kept down to about half the usual maximum dose, but the tendency to vomit can frequently be overcome partially by increasing the dose very cautiously. Vomiting after the injection of Neostam frequently occurs, and constitutes a serious drawback to the use of this compound.

The patient usually vomits within about 20 minutes of receiving the injection; vomiting may be preceded by giddiness and nausea. If, when these premonitory symptoms appear, the patient remains quietly in bed the vomiting may be avoided; an injection should not be given immediately after the patient has taken a meal.

Diarrhœa.—One or two patients have been attacked by a severe diarrhœa towards the end of a course of treatment. The motions are very frequent; they are dark and watery, but do not contain blood or mucus; the diarrhœa is usually accompanied by vomiting. The patient becomes collapsed and the condition is usually precarious—one of the author's patients died—but if the treatment is discontinued the condition usually improves again.

Anaphylactic-like syndrome.—This group of symptoms usually occurs quite suddenly after the sixth or the seventh injection, when the patient has been receiving the maximum dose for the last few injections; that is to say, it is usually not associated with an increase in the dose administered. Within a few minutes of the giving of the injection the patient's face becomes puffy and an urticarial rash appears all over the body; the voice becomes husky and there is considerable difficulty in breathing; the patient is collapsed, the pulse being imperceptible at the wrist; or the collapse may be accompanied by violent diarrhœa and vomiting, and the patient becomes cyanosed, breathes stertorously, and remains unconscious for some minutes. All the symptoms usually disappear within two hours, but the puffiness of the face may remain for 24 hours.

Most of the cases reported were receiving injections of urea-stibamine or Aminostiburea (Napier 1926*c*). A number of the present writer's patients have shown a mild degree of these symptoms; the majority of these were being treated with Aminostiburea.

These symptoms are very alarming, but no deaths have been reported. As further administration of the smallest dose may lead to a recurrence of these symptoms, it is best to abandon treatment with the particular compound altogether. In the writer's cases the course of treatment was almost completed and no further injections were given, but in cases in which further treatment is considered necessary it would be best to recommence treatment with minute doses of some other compound.

Hepatitis.—In a few instances, during treatment with Stibosan and urea-stibamine, the patient has developed symptoms of acute congestion of the liver; that organ becomes markedly enlarged and the patient complains of severe pain in the hepatic region; there is a return of fever, if the temperature had already fallen to normal;

the patient becomes drowsy and there is slight jaundice. If the treatment is discontinued immediately the symptoms will usually subside.

Relapses

A relapse after insufficient treatment is by no means uncommon. It is repeatedly stated that a patient who has been treated previously, and in whom the condition has relapsed, requires a bigger dose of antimony to cure his disease than he would have required in the first instance; or, in other words, that the disease has become antimony resistant. There is little evidence to support this contention, and in the present writer's opinion it is not the case. In some patients the disease is much more resistant to treatment than in others; according to the graph on page 169, if 100 persons were given a moderate course of treatment with Stibosan, of, say, 3 grammes per 100 lbs. body-weight, 13 of them would relapse. If these 13 patients now come under treatment, and were given a more severe course, equivalent to 4·0 grammes per 100 lbs. body-weight (according to this graph), we should still expect seven of them, i.e. more than 50 per cent., to relapse. It is this high relapse rate amongst patients that have previously relapsed which has led to the erroneous impression that it is the previous treatment that has made the disease more resistant.

It is obvious, therefore, that when a relapse occurs the patient must be given a further and much more thorough course of treatment. If, however, the symptoms reappear within a short period of, say, a month or six weeks, and the patient is again put under treatment immediately, a course equal to the first course will often be sufficient to complete the cure.

It must be remembered that patients who have had kala-azar are not immune from fever from other causes, and care should be taken not to diagnose fever due to malaria, dengue or influenza as a relapse of kala-azar; the patient will be only too ready to help you to make this error.

A very definite enlargement of the spleen usually accompanies fever that is due to a relapse, but where possible a spleen puncture should be done to confirm the diagnosis, as other diagnostic points may be overshadowed. If a period of some months has elapsed it is usually quite easy to decide the question, and in this case the aldehyde test will again be of value.

Whenever a relapse has occurred after a course of sodium antimony tartrate, one of the more powerful antimonials should, if possible, be substituted in the second course of treatment.

TREATMENT OF PREGNANT WOMEN

Treatment should certainly not be withheld on account of pregnancy. This complication, on the other hand, is one that makes it imperative that immediate steps should be taken to treat the disease. At whatever stage of pregnancy the patient is seen, treatment should be commenced without delay. The writer has treated a number of cases of pregnant women, who have subsequently gone to full time and given birth to healthy children.

TREATMENT BY INTRAMUSCULAR INJECTIONS

This method of treatment is sufficiently important to demand special reference.

ADVANTAGES

Were it possible to find a compound that would cause no pain on intramuscular injection and at the same time would possess curative powers, when given by this method, equal to that of, say, Stibosan intravenously injected, a great advance in the treatment of kala-azar would have been made. The advantages of this method of introducing antimony are obvious. A certain amount of skill is required in giving an intravenous injection to a patient; an inexperienced practitioner may cause a patient a considerable amount of unnecessary pain. There are a number of practitioners who are unable or unwilling to give intravenous injections; consequently large numbers of patients are doomed to die simply because they are not within reach of sufficiently skilled medical assistance. The practitioner with the poorest qualifications is capable of giving a subcutaneous injection, even though he may not give it with the maximum degree of asepsis, and could easily be shown, by written instructions, how to give an intramuscular one.

Furthermore, there are a number of very young children and a few adults in whom the veins are so small, or so deeply buried in fat, that it is almost impossible for even the most experienced to puncture them.

PREPARATIONS

For this purpose a very large number of compounds of antimony have been tried, and an even larger number suggested, but so far no real success can be claimed. Some preparations cause considerable pain at the time of injection with a little thickening of the tissues afterwards, others cause less pain at the time with considerable swelling and pain afterwards, and yet others cause no pain but have no therapeutic effect.

If great care be observed in giving the injection strictly intramuscularly, most of the preparations which are in use can be administered in small doses without much pain. A number of children have been cured by repeated injections of 0·1 gramme of Stibosan into the gluteal region. Larger doses than this usually give rise to pain and swelling, but, if the injections are given into a different place each time, this dose can be given repeatedly. There is a certain amount of pain at the time of injection. This pain will frequently not be so well marked at the beginning of the treatment, but as the treatment progresses the pain becomes more noticeable. It is not, however, unbearable, and usually only lasts a few minutes. A slightly tender lump may be left at the site of injection for a few days, but there is never the red and painful swelling that occurs if the ordinary antimony tartrate is given subcutaneously.

DOSAGE

As stated above, small doses only should be administered by this route, 0·1 gramme being the maximum that it is advisable to give, that is to say, two cubic centimetres of a five per cent. solution. For a child this dose may be sufficiently large to effect a cure in ten to twelve injections, but for an adult, in order to reach the required total dose, it may be necessary to give daily injections and to give at least 25 of them.

DETAILS

The best site for injections is at any point along a line drawn parallel to and one inch below the crest of the ilium. The reader might be reminded of the old rule for giving intramuscular injections, that is, if a patient is placed in a sitting position on a stool, the injection can be given to any part of the buttock that is

accessible without fear of causing any damage to important structures. Care should be taken that the injection is not given subcutaneously, as in this case much more pain will be caused. If, on the other hand, it is given too deeply, that is, against the bone, very severe pain will be felt down the leg. A little gentle massage will sometimes ease the pain and will hasten absorption.

CONCLUSIONS

Up to the present no entirely satisfactory compound for intramusular injection has been found; the above-described method must only be resorted to in cases where the intravenous route is not possible, but under these circumstances it should never be neglected.

SUBSIDIARY TREATMENT

It is well known that most irritants that cause an acute inflammation, be they organic, mechanical, chemical or physical, tend to produce an increase in the leucocytes, and especially in the number of polymorphonuclear leucocytes. It has also been pointed out that certain inflammatory processes, such as, pneumonia, dysentery and cancrum oris, often appear as complications of kala-azar; in these complications there is an increase in the previously diminished number of polymorphonuclear leucocytes, generally accompanied by a shrinking in the size of the liver and spleen, and, where the process is arrested in time, by a general improvement in the condition of the patient. At the same time it is these very complications which are so often the cause of death.

COUNTER IRRITANTS

One of the first ideas in the treatment of kala-azar was to forestall these complications, and to produce leucocytosis by the local application of some irritant. This method of treatment has long been resorted to by the uneducated inhabitants of various Eastern countries. In Syria a seton is applied in the form of a string tied in a loop round a piece of skin above the spleen. A chronically inflammed sinus is thus kept up indefinitely. Among the Santhals cauterisation over the spleen with a red-hot instrument is a common form of treatment. Among Bengalis counter-irritation is carried out by the application of heat and the rubbing

on of fig leaves. Certain drugs, when injected intramuscularly, have the effect of producing leucocytosis, for example soamin, streptococcal vaccines and turpentine.

Muir (1918) cured a number of patients by the injection of turpentine; he was in communication with some of these for upwards of six years, during which time they remained entirely free from the disease.

T.C.C.O.—Muir has suggested the following as a more convenient and safe leucocyte producer: Turpentine, 1 drachm; camphor, 1 drachm; creosote, 1 drachm; olive oil, 2½ drachms. The solution, being strongly antiseptic, is very safe, but should not be kept for more than a month. A dose should be given intramuscularly, in the gluteal region, for preference, sufficient to produce local swelling and pain, but not sufficient to form an abscess. As an initial dose, 0·5 cc. should be given into each buttock; the second injection can be larger or smaller, according to the reaction produced. If an abscess forms through an overdose it is an aseptic abscess, and the pus may be evacuated by aspiration or by making a small puncture with the point of a sharp knife. If it be evacuated in time it will heal up at once.

In an acute case, where the general condition of the patient is bad, it is often as well to withhold the antimony injections and to give a few preliminary injections of *T.C.C.O.*

In cases which have hard fibrous spleens, and in which the temperature during the course of treatment remains obstinately at about 100 degrees, an injection or two of *T.C.C.O.* will often cause a sharp reactionary rise to about 103 degrees for a few days, followed by a fall to normal, and at the same time considerable softening and diminution in the size of the spleen. It is very important that the injections of antimony should be continued when the *T.C.C.O.* is being given.

GENERAL

It is a good plan to put any patient who is at all debilitated on to a digitalis mixture of some kind. This will usually steady down the pulse rate, but if the pulse rate becomes unduly slow, as it does in a few cases, the digitalis must be discontinued.

For an adult the following, given three or four times daily, will be found useful:

℞

Tincturæ Digitalis	m.	XV
Tincturæ Nucis Vomicæ	m.	V
Tincturæ Rhei Compositæ	m.	XX
Tincturæ Cardamoni Compositæ	m.	XV
Aquam Chloroformi ad.	oz.	½

When the patient is being treated in, or has come from, a malarious district, as is often the case, it is important that quinine should be taken daily, otherwise he is very liable to have a malarial attack whilst under antimony treatment.

Although as a rule a general tonic is not essential, nor, in the opinion of the writer, even advantageous, the following prescription will be found useful, especially in the cases, as indicated above, where it is thought advisable to give quinine.

℞

Ferri et Quininæ Citratis	grs.	V
Quininæ Sulphatis	grs.	IV
Sodii Sulphatis	grs.	XXX
Acidi Hydrochlorici diluti	min.	III
Liquoris Arsenici Hydrochlorici	min.	III
Syrupi Aurantii	min.	XX
Aquam ad	oz.	½

Take three doses daily after food.

When the course of injections is completed it is nearly always advisable to prescribe some tonic. For this purpose the above prescription, with or without the quinine sulphate, or one of the proprietory tonics, such as syrup of hæmoglobin, may be given. For children, syrup of hypophosphates, or, if the weather is cold, malt and cod-liver oil, will be found useful.

Diet

It is unnecessary to enter in detail into the hygienic conditions necessary for a kala-azar patient, as they do not differ from those required by a patient suffering from any similar disease. It is not possible to lay down any hard and fast rules as to how long a patient should remain in bed, but it is safe to say that, if it is at all possible, the time should be at least a fortnight to three weeks, by which time the majority of patients will have made their first steps towards recovery.

The question of diet is an extremely difficult one. Strictly speaking, there is no reason why the patient who is getting little

fever, has a good digestion and is not suffering from diarrhœa should not eat whatever he likes; a very large number do; but if one allows this freedom of diet there is always a grave danger of a patient, whose appetite becomes voracious as his general condition improves, seriously over-eating. It is, therefore, better to advocate caution, even at the risk of giving the patient a little unnecessary discomfort.

Europeans should take a full milk diet, i.e. milk *ad lib*, milk-puddings, bread and butter, toast, chicken broth, grapes, or other fruit juice and vegetable juices, as long as the fever is above 100° F. daily. Fish, chicken, eggs and a few vegetables can be added to this as the temperature becomes normal.

Indian patients should be advised to substitute sago and milk for the boiled rice of their normal meal, to drink milk and fruit juice, and to avoid all curries, highly spiced dishes and heavy fruits. It will probably be found advisable at an early stage of the treatment to allow one meal of rice boiled with milk, if possible, and dhal. Allow them gradually to get on to their full diet, but always warn them against the dangers of over-eating at any single meal. Practically the only reason for which a hospital patient ever demands to be discharged before his treatment is completed is that he is not getting sufficient food, and yet he will probably be having a full hospital diet, not an ungenerous diet, and a number of 'extras'.

The Treatment of Complications

Most of the common complications are treated on general lines, but a few notes on the special treatment of one or two of the commoner complications might be of some assistance.

DYSENTERY

Strict dieting (arrowroot and whey) and rest in bed should be the first considerations. If no specific organism has been found, it is best to commence with four-hourly doses of two drachms of castor oil, and to follow this with astringent treatment, as, for example, repeated doses of Pulvis cretæ aromaticus cum opio, grains X. Magnesium sulphate should be avoided, and if salts are prescribed sodium sulphate should be given.

Dimol will be found useful in this condition.

CANCRUM ORIS

There is usually little hope of curing this disease by local measures, the only hope being to keep the local condition *in statu quo* until the general condition of the patient improves. The antimony treatment must not be discontinued. In the early stages an alum mouth wash:

R

Alum	grs.	LXXX
Tincturæ Myrrhæ	oz.	II
Aquam ad	oz.	VIII

should be used very frequently during the day. The sloughs should be removed as soon as possible, and the ulcer irrigated with weak eusol. Strong antiseptics should be avoided, as the tissues are very poorly resistant and may easily be killed. A more heroic form of treatment, but, as the condition frequently proves fatal, a perfectly justifiable one, is to attempt to limit the spread of the disease by burning with the thermo-cautery a deep ring well outside the diseased area.

Polyvalent streptococcal serum is usually of some value in this condition.

MALARIA

Plasmodial and leishmania infections must co-exist in the same patient very frequently, but the occasions on which both parasites will be found in the same film of either peripheral blood or spleen puncture material are very few. In the same way, malarial attacks are uncommon when a patient is suffering from kala-azar, but once this disease is under control with antimony treatment a typical attack of malaria is not uncommon. An attack of this kind has a very depressing effect on the patient, who immediately imagines that he is getting a return of his kala-azar. Furthermore, at the end of a course of treatment, when the temperature has returned to normal for a few days, it occasionally rises again to the 100° mark on alternate days, or in some instances every day; in these cases malarial parasites may be found in the peripheral blood, but whether they are, or are not, found, this residual temperature almost always disappears when quinine, or one of the cinchona alkaloids, is administered. A suitable prescription for an adult is the following:

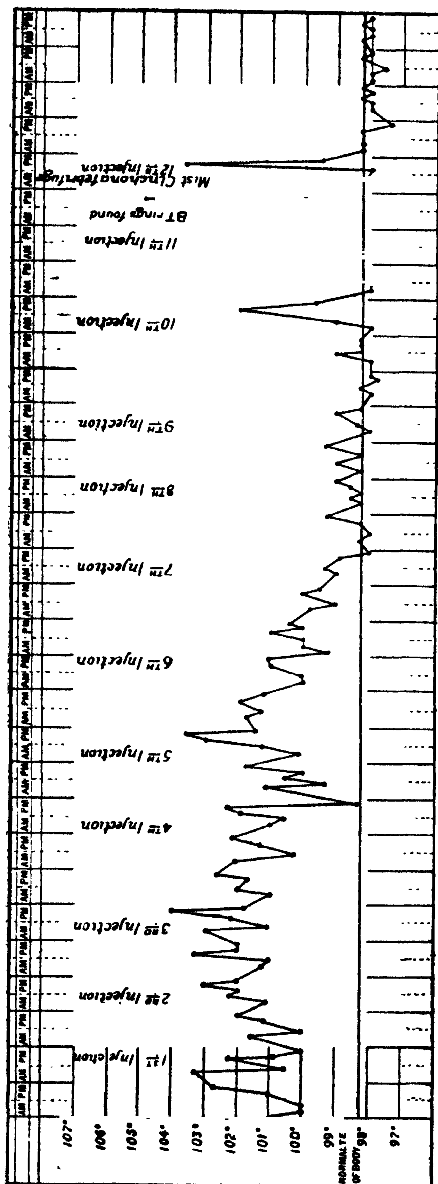


CHART XV

Four-hourly temperature record in a case in which kala-azar was complicated by malaria. Treatment with a pentavalent compound of antimony was given; the reaction to treatment appeared to be delayed, then typical malarial rigors appeared.

℞

Cinchona Febrifuge	.. grs.	X
Acidi Citratis	.. grs.	XV
Magnesii Sulphatis	.. grs.	XXX
Sodii Sulphatis	.. grs.	XXX
Syrup	.	q. s.
Aquam Chloroformi ad	.. oz.	I

This mixture should be taken twice daily, about two hours after a meal.

ANKYLOSTOMIASIS

In Bengal, where the disease is very common, practically every Indian kala-azar patient has either a severe or a mild hookworm infection. It is extremely important for the patient that this condition should be treated, as the return of the blood picture to normal can hardly be expected as long as this complication is allowed to remain. If the condition of the patient is good, it is as well to undertake this treatment immediately, but if he is very debilitated, or if there is a very decided tendency towards diarrhœa, it will probably be better to withhold the anthelminthic measures until the condition of the patient has improved. The patient should be given 60 minims of carbon tetrachloride emulsified in two ounces of milk, ten to fifteen minims of oil of chenopodium in capsules, and one ounce of sodium sulphate in solution at one time. Milk is the best vehicle for carbon tetrachloride, but if it is inconvenient to give milk the drug can be mixed with the salt solution. If the original infection was a heavy one, it will always be advisable to examine the stools of the patient about one month after the initial dose has been given, and, if necessary, repeat the treatment.

THE TREATMENT OF THE COMPLICATIONS ASSOCIATED WITH THE ANTIMONY INJECTIONS

The most important point is to discontinue the injections or to reduce the dose, according to the severity of the symptoms. Acute symptoms, such as vomiting, usually pass off without any treatment being given. Diarrhœa should be treated by strict dieting and the administration of bismuth and opium. Swelling of the face and dyspnœa are usually relieved immediately by the administration of adrenalin, 15 minims, and, if necessary, strychnine and digitalin can be given to combat the collapse.

The acute congestion of the liver that occurs in some cases should be taken as a serious complication; antimony treatment must be discontinued immediately. The pain will be relieved by a counter-irritant, such as a belladonna plaster; and aspirin and calomel in small repeated doses should be given.

The post-treatment jaundice does not call for any special treatment. The patient should be told to drink large quantities of fluid, if possible some medicinal water, such as Vichy. Calomel in small doses and saline purgatives should be given daily.

Prognosis

Prior to the introduction of antimony in the treatment of kala-azar the death-rate was reported to be 95 per cent. by some observers, but others placed it as low as 75 per cent. of clinically definite cases. Undoubtedly, a large number of persons suffer from mild attacks in which spontaneous cure occurs. We have no means of ascertaining what proportion of these infected persons develop clinical kala-azar; it is therefore difficult to make any estimate of the cure rate amongst untreated cases.

Probably 85 per cent. to 90 per cent. of all patients that come under suitable treatment recover.

Amongst patients treated with one of the more successful pentavalent compounds the eventual cure rate is possibly 95 per cent.

In a small percentage, probably less than one per cent., of patients that come under treatment the disease proves totally resistant to antimony in any form whatsoever. These patients are usually not acutely ill; they sometimes live for a year or two, and then die very suddenly.

The prognosis is poor in cases in which there is cancrum oris, or in which the patient is exceedingly emaciated, and is very bad in cases in which there is marked ascites due to fibrotic changes in the liver.

Treatment of Dermal Leishmaniasis

The intravenous injection of antimony, in some form or another, would appear to be the only treatment that has any effect on the lesions in this condition. The cases in which there is no history of the patient having had kala-azar and of having been treated



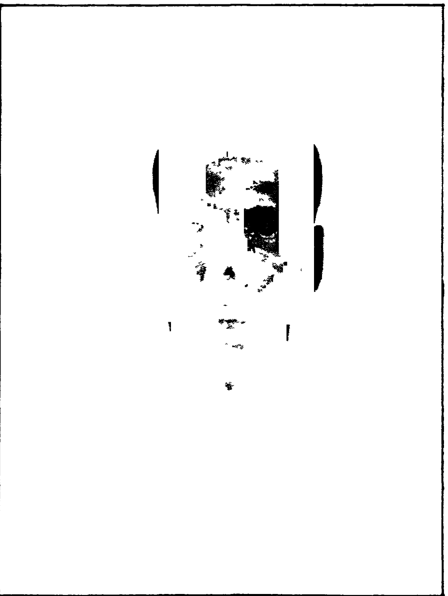


Plate XVIII

Dermal Leishmaniasis, advanced second stage: before and after treatment.

for it, clear up rapidly; most of the cases that the writer has seen have been treated with Stibosan. The nodules disappear after about ten injections, and there does not appear to be any tendency to recurrence after the treatment is discontinued.

The cases in which there is a history of previous treatment for kala-azar may prove very refractory to treatment. In one case more than 20 injections of Stibosan, over 5 grammes of the preparation, only produced very slight improvement; antimony ionisation, X-ray exposures, and large doses of iodides by the mouth, were all tried with little result.

Summary of Treatment

A certain diagnosis should precede treatment. Where there is doubt as to the diagnosis it is difficult to proceed with the treatment steadfastly enough to effect a cure, and it may be difficult to confirm the diagnosis after treatment has continued for some time.

There are two alternatives offered for the treatment of the disease, namely, treatment with the pentavalent or with the tri-valent compounds of antimony. There is no doubt that treatment with one of the pentavalent compounds of proved efficacy is the treatment of choice; there is only one point against these compounds, and that is their high cost. If cost is not a matter of consideration, then one of the pentavalent compounds should be used, but, as there may be occasions when large numbers have to be treated and the cost of the preparation has to be considered, both forms of treatment will be dealt with.

If Stibosan be used dosage will be as follows:

Injections should be given intravenously thrice a week. A freshly prepared five per cent. solution, in sterile distilled water, should be used. The initial dose for an adult should be 0·2 gramme and subsequent doses of 0·3 grammes; a child up to the age of fourteen years will stand 0·25 gramme; a child of ten years, 0·2 gramme; a child of six years, 0·15 gramme; and a child of two years, 0·1 gramme, as a maximum dose. The injections should be given until the patient has received a total dose equivalent to 3·5 grammes per 100 lbs. weight of body. The only contra-indications to treatment with these compounds are vomiting or severe diarrhœa, which are indications for the reduction of the dose, and hepatitis, which may necessitate the discontinuance of the injections altogether.

The details of dosage for the other pentavalent compounds are very similar.

If circumstances necessitate the use of antimony tartrates the following are the details of treatment:

A carbolised or freshly prepared two per cent. solution of a pure sample of sodium antimony tartrate should be injected intravenously three times a week. In the case of an adult, commence with a dose of about 0·04 gramme (2 cc.), but if the patient is very weak a smaller dose should be given. Increase the dose by 0·02 gramme up to a maximum of 0·1 gramme, or, if the patient cannot tolerate this dose, up to the point of tolerance. Signs of intolerance are too great a febrile reaction—more than two degrees—lung symptoms—such as violent coughing, bronchitis or pneumonia—vomiting, severe diarrhœa or dysentery. When any of these symptoms appear, either the dose at the next injection must not be increased, or it must be reduced, or the antimony tartrate injections must be suspended and some other form of antimony administered, according to the severity of the symptoms.

It is better to continue the injections for too long a period than to stop them too soon, provided the patient stands them well, as do the majority of patients; a relapse means that another full course of treatment will have to be given. Four grammes of the salt per 100 lbs. body-weight of patient should be considered the full course of treatment, which in certain cases—when the temperature reacts early to treatment, the spleen shows a marked reduction, the weight has increased and the leucocyte count is above 6,000 per c.mm.—can be reduced, but at least 30 injections should be given in every case.

Patients that are not cured by this course of treatment are not suitable for treatment with the antimony tartrates, and should be given injections of one of the pentavalent compounds of antimony.

Where there is a hard fibrous spleen, or a spleen which does not diminish rapidly under antimony injections, then intramuscular injections of T.C.C.O. should be given. When T.C.C.O. is given the injections of antimony *should not be discontinued* during the resulting reaction.

When the patient has previously suffered from malaria, comes from a malarious district, or is being treated in a place where malarial infection is common, it is well to give quinine in preventive doses during the period of specific kala-azar treatment.

Where the blood pressure is low, especially at the beginning of treatment, digitalis should be administered.

Diarrhœa or dysentery should, after a few doses of castor oil, be controlled by astringents, such as Pulvis cretæ aromaticus cum opio, grs. X, after each excessive motion, and the antimony continued cautiously. The bowel complaint, if controlled, will tend to disappear as the kala-azar improves. Where, on the other hand, there is evidence of amœbic infection of the bowel suitable specific treatment should be given.

Bronchial or broncho-pneumonic complications should be treated with mild expectorants and heroic counter-irritation of the chest.

Remembering the blood condition, blood tonics, such as iron in the form of syrup of hæmoglobin and arsenic, should be given. The bowels should be kept freely open with laxatives when necessary.

Only light diet should be given until the temperature becomes normal, and the patient should be warned against excessive eating. The excessive appetite common during recovery tends to produce over-eating, which may be followed by serious bowel complications.

REFERENCES TO LITERATURE

ACTON, H. W. and NAPIER, L. EVERARD (1927).—Post-kala-azar dermal leishmaniasis.—*Indian Journ. Med. Res.*, Vol. XV, No. 1, in press.

ADIE, HELEN A. (1921).—A preliminary note on the development of the Leishman-Donovan parasite in the spleen juice and in the alimentary tract of *Cimex lectularius* (Lin.).—*Indian Journ. Med Res.*, Vol. IX, No. 1, p. 255.

ARCHIBALD, R. G. (1914).—A preliminary report on some further investigations on kala-azar in the Sudan.—*Journ. Roy. Army Med. Corps*, Vol. XXIII, No. 5, p. 485.

AWATI, P. R. (1922).—Survey of the biting insects of Assam with reference to kala-azar.—*Indian Journ. Med. Res.*, Vol. X, No. 2, p. 579.

BANERJEE, K. L. (1925).—Report quoted by Brahmachari in *Kala-Azar and Its Treatment*, 2nd ed., enlarged. Butterworth, Calcutta.

BARRAUD, P. J. (1926).—Report upon a sandfly survey of Madras Town. —*Indian Journ. Med. Res. Memoirs*, No. 4, p. 207.

BASILE, C. (1911)—Sulla Leishmaniosi e sul suo modo di transmissione.—*Rendiconti R. Accad dei Lincei*, March, p. 479 ; April, p. 590 ; June, p. 955 ; July, p. 72.

BASU, S. C. (1924).—Age incidence of kala-azar patients.—*Indian Med. Rec.*, Vol. XLIV, No. 4, p. 107.

BENTLEY, C. A. (1902).—Epidemic Malta fever in Assam.—*Indian Med. Gaz.*, Vol. XXXVII, p. 337.

— (1924).—*Bengal Public Health Report for 1922.* Bengal Secretariat Book Depot, Calcutta.

BOUSFIELD, L. (1911).—Remarks on kala-azar in the Kassala and Blue Nile districts of the Sudan.—*Fourth Report of the Wellcome Research Laboratories*, Vol. A, p. 127.

BRAHMACHARI, U. N. (1917).—Fourth Report on the treatment of kala-azar and some blood reactions in this disease.—*Indian Med. Gaz.*, Vol. LII, No. 9, p. 319.

— (1920).—*Kala-azar and Its Treatment.* 2nd edition. Butterworth, Calcutta.

— (1922).—A new form of cutaneous leishmaniasis.—*Indian Med. Gaz.*, Vol. LVII, No. 4, p. 125.

— (1922*a*).—The chemotherapy of antimonial compounds in kala-azar infection : Part I, *Indian Journ. Med. Res.*, Vol. X, No. 2, p. 492.

— (1923).—The chemotherapy of antimonial compounds in kala-azar infection : Part IV.—*Indian Journ. Med. Res.*, Vol. XI, No. 2, p. 393.

CARONIA, G. (1916).—Limpiego di nuovi preparati di antimonio per via intramuscolare nella cura della leishmaniosi.—*Pediatria*, Vol. XXIV, No. 2, p. 65.

CHOPRA, R. N. (1926).—A preliminary note on the pharmacological action of certain organic antimony derivatives.—*Indian Med. Gaz.*, Vol. LXI, No. 4, p. 162.

— (1927).—An experimental investigation into the action of organic compounds of antimony.—*Indian Journ. Med. Res.*, Vol. XV, No. 1, in press.

CHRISTOPHERS, S. R. (1904).—On a parasite found in persons suffering from enlargement of the spleen in India.—*Scientific Memoirs*, New series, No. 11, Government of India Printing, Calcutta.

— (1926).—Introduction.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 3.

CLARK (1882).—Report of the Sanitary Commissioner of Assam.

COCHRANE, S. (1913).—The superficial lymph nodes as a source of leishmania for the diagnosis of kala-azar; with some observations on kala-azar in China.—*Journ. London Sch. Trop. Med.*, Vol. II, p. 179.

CORNWALL, J. W. and LA FRENAIS, H. M. (1916).—A contribution to our knowledge of kala-azar.—*Indian Journ. Med Res.*, Vol. III, No. 3, p. 698.

CORNWALL, J. W. (1916).—A contribution to the study of kala-azar.—*Indian Journ. Med. Res.*, Vol. IV, No. 1, p. 105.

DI CRISTINA, G. and CARONIA, G. (1912).—*Pathologica*, September 1st.

— (1915).—Sulla terapia della leishmaniosi interna.—*Pathologica*, Vol. VII, No. 151, p. 82; and *Pediatria*, Vol. XXIII, No. 2, p. 81.

CRITIEN, A. (1910).—Kala-azar infantile â Malte.—*Archiv. de l'Instit. Past. de Tunis.*, Vol. II, p. 49.

CUNNINGHAM, J. and VARADARAJAN, P. S. (1923).—Kala-azar in Madras Presidency outside Madras City.—*Indian Med. Gaz.*, Vol. LVIII, No. 7, p. 308.

CUNNINGHAM, J. and PUNDIT, S. R. (1925).—A new endemic focus of kala-azar in Southern India.—*Indian Journ. Med. Res.*, Vol. XII, No. 4, p. 743.

DONOVAN, C. (1903)—On the possibility of the occurrence of trypanosomiasis in India.—*British Med. Journ.*, Vol. II, p 79.

— (1913).—Kala-azar, its distribution and probable modes of infection.—*Indian Journ. Med. Res.*, Vol. I, No. 1, p. 177.

FANTHOM, H. B. and PORTER, A. (1915).—Insect flagellates and the evolution of disease.—*Ann. Trop. Med. and Parasit.*, Vol. IX, p. 335.

FARGHER, R. G. and GRAY, W. H. (1921).—The chemotherapy of antimony.—*Journ. Pharm. and Exp. Therap.*, Vol. XVIII, p. 341.

FOSTER, P. (1924).—Urea-stibamine in the treatment of kala-azar under tea garden conditions.—*Indian Med. Gaz.*, Vol. LIX, No. 8, p. 391.

FRANCA, C. (1920).—La flagellose des euphorbes.—*Ann. de Inst. Past.*, Vol. XXXIV, p. 432.

FRANCHINI, G. (1912).—On the presence of leishmania in the digestive tract of *Anopheles maculipennis*.—*Ann. Trop. Med. and Parasit.*, Vol. IV, No. 1, p. 41.

GABBI, U. (1911).—Pulci canina et umana non propagano il kala-azar.—*Malaria e Malattie dei Paesi Caldi*, October, p. 285.

GILES, G. M. (1892).—Notes on ankylostomiasis.—*Indian Med. Gaz.*, Vol. XXVII, No. 5, p. 170.

GREIG, E. D. W. and CHRISTOPHERS, S. R. (1925).—The infection of a monkey (*Macacus rhesus*) with the parasite of Indian kala-azar.—*Indian Journ. Med. Res.*, Vol. XIII, No. 1, p. 151.

GREIG, E. D. W. and KUNDU, S. (1925).—Observations on the quantity of ureastibamine, von Heyden 471, and stibamine glucoside required for the complete treatment of a kala-azar case.—*Indian Journ. Med. Res.*, Vol. XII, No. 4, p. 679.

DAS GUPTA, B. M. (1922).—The diagnosis of kala-azar by peripheral blood culture (abstract).—*Indian Med. Gaz.*, Vol. LVII, No. 6, p. 217.

HOARE, C. A. (1921).—Some observations and experiments on insect flagellates. —*Parasitology*, Vol. XIII, No. 1, p. 67.

KALA-AZAR COMMISSION :

CHRISTOPHERS, S. R., SHORTT, H. E. and BARRAUD, P. J. (1925).—Culicoides and kala-azar in Assam.—*Indian Journ. Med. Res.*, Vol. XIII, No. 1, p. 175.

— (1925*a*).—The effect of salt solutions of different concentrations on the parasite of Indian kala-azar.—*Indian Journ. Med. Res.*, Vol. XIII, No. 1, p. 177.

— (1926).—Morphology and life cycle of the parasite of Indian kala-azar in culture.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 19.

— (1926*a*).—The result of intra-peritoneal inoculation of mice with cultures of *Leishmania donovani*.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 77.

— (1926*b*).—The result of feeding mice on cultures of *Herpetomonas donovani*.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 89.

— (1926*c*).—The development of the parasite of Indian kala-azar in the sandfly, *Phlebotomus argentipes*, Annandale and Brunetti.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 123.

— (1926*d*).—Further observations on the feeding of sandflies, *Phlebotomus argentipes*, on cases of Indian kala-azar.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 127.

— (1926*e*).—Development of the parasite of Indian kala-azar in the sandfly, *Phlebotomus argentipes*; controls.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 135.

— (1926*f*).—The feeding of larvæ of *Phlebotomus argentipes* on cultures of *Leishmania donovani*.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 155.

— (1926*h*).—The anatomy of the sandfly, *Phlebotomus argentipes*, Ann. and Brun.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 177.

SHORTT, H. E., BARRAUD, P. J. and CRAIGHEAD, A. C. (1926*g*).—A note on the massive infection of the pharynx of *Phlebotomus argentipes* with *Herpetomonas donovani*.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 157.

SHORTT, H. E., BARRAUD, P. J. and CRAIGHEAD, A. C. (1926*i*).—The life history and morphology of *Herpetomonas donovani* in the sandfly, *Phlebotomus argentipes.—Indian Journ. Med. Res.*, Vol. XIII, No. 4, p. 947.

— (1926*j*).—Note on the massive infection of the buccal cavity of *Phlebotomus argentipes* with *Herpetomonas donovani.—Indian Journ. Med. Res.*, Vol. XIV, No. 2, p. 329.

— (1926*k*).—The finding in nature of *Phlebotomus argentipes* infected with a flagellate identical with *Herpetomonas donovani.—Indian Journ. Med. Res.*, Vol. XIV, No. 2, p. 505.

KNOWLES, R. (1920).—A study of kala-azar.—*Indian Journ. Med. Res.*, Vol. VIII, No. 1, p. 140.

KNOWLES, R. and DAS GUPTA, B. M. (1924).—The diagnosis of kala-azar by the examination of thick films.—*Indian Med. Gaz.*, Vol. LIX, No. 9, p. 438.

KNOWLES, R., NAPIER, L. E. and DAS GUPTA, B. M. (1923).—The kala-azar transmission problem.—*Indian Med. Gaz.*, Vol. LVIII, No. 7, p. 321.

KNOWLES, R., NAPIER, L. E. and SMITH, R. O. A. (1924).—On a herpetomonas found in the gut of the sandfly, *Phlebotomus argentipes*, fed on kala-azar patients.— *Indian Med. Gaz.*, Vol. LIX, No. 12, p. 593.

LAVERAN, A. (1917).—*Leishmanioses* —Masson et Cie, Paris.

LEISHMAN, W. B. (1903).—On the possible occurrence of trypanosomiasis in India.—*British Med. Journ.*, Vol. I, p. 1252.

LLOYD, R. B., NAPIER, L. E. and SMITH, R. O. A. (1925).—The blood meal of *Phlebotomus argentipes* identified by precipitin antisera.—*Indian Journ. Med. Res.*, Vol. XII, No. 4, p. 811.

LOW, G. C. and COOKE, W. E. (1926).—A congenital case of kala-azar.—*Lancet*, Vol. CCXI, p. 1209.

MACKIE, P. F. (1914).—Bodies of unknown nature found in the fæces of kala-azar patients.—*Indian Journ. Med. Res.*, Vol. II, No. 2, p. 510.

— (1915).—Insects and kala-azar.—*Indian Journ. Med. Res.*, Vol. II, No. 4, p. 942.

— (1916).—The presence of leishmania in the peripheral blood of kala-azar cases in Assam.—*Indian Journ. Med. Res.*, Vol. III, No. 1, p. 90.

— (1921).—Correspondence.— *Indian Med. Gaz.*, Vol. LVI, No. 2, p. 76.

MACKIE, P. F., DAS GUPTA, B. M. and SWAMINATH, S. C. (1923).—Progress report on kala-azar.—*Indian Journ. Med. Res.*, Vol. XI, No. 2, p. 591.

MANSON, P. (1919).—*Tropical Diseases*, 6th edition.—Cassell, London.

MANSON-BAHR, P. (1920).—The intravenous use of acetyl-amino-phenyl salt of antimony (Stibenyl) in the treatment of human trypanosomiasis and kala-azar.—*Lancet*, Vol. CXCIX, p. 178.

MARIAN-PERRY, H. (1922).—*L. donovani* in the intestinal tissues.—*Journ. Roy. Army Med. Corps*, Vol. XXXIX, No. 5, p. 323.

MARSHALL, W. E. (1911).—Report of the Kala-azar Commission to investigate the prevalence and cause of this disease in the Eastern Sudan:

Pathological report.—*Fourth Report of the Wellcome Research Laboratories*, Vol. A, p. 157.

MARTIN and LE BOEUF (1908).—Sur le traitement de la trypanosomiase humaine.—*Bull. Soc. Path. Exot.*, Vol. I. p. 508.

MCVAIL, J. B. (1922).—The blood count in ankylostomiasis.—*Indian Med. Gaz.*, Vol. LVII, No. 10, p. 366.

MELENEY, H. E. (1925).—The histopathology of kala-azar in the hamster, monkey and man.—*American Journ. Path.*, Vol. I, No. 2, p. 147.

MUIR, E. (1915).—The treatment of kala-azar with tartar emetic.—*Indian Med. Gaz.*, Vol. L, No. 10, p. 365.

— (1918).—*Kala-azar ; Its Diagnosis and Treatment.*—Butterworth, Calcutta.

NAPIER, L. EVERARD (1921).—A new serum test for kala-azar.—*Indian Med. Gaz.*, Vol. LVI, No. 9, p. 338.

— (1922).—A preliminary note on the treatment by the intramuscular injection of a special preparation of sodium antimony tartrate.—*Indian Med. Gaz.*, Vol. LVII, No. 1, p. 10.

— (1922*a*).—A new serum test for kala-azar.—*Indian Journ. Med. Res.*, Vol. IX, No. 4, p. 830.

— (1922*b*).—The differential blood count.—*Indian Med. Gaz.*, Vol. LVII, No. 5, p. 176.

— (1922*c*).—A report on the treatment of 10 cases of kala-azar by sodium acetyl-para-amino-phenyl stibiate.—*Proc. Roy. Soc., Med.*, Vol. XV, Part III, p. 44 ; and *Lancet*, Vol. CCIV, p. 282, 1923.

— (1922*d*).—An analysis of the clinical picture in kala-azar.—*Indian Med. Gaz.*, Vol. LVII, No. 11, p. 406, and No. 12, p. 446.

— (1923).—Report of the work of the kala-azar laboratory.—*Ann. Report for 1922, Calcutta Sch. Trop. Med. and Hyg.*, p. 21.

— (1923*a*).—Further experience with the aldehyde reaction.—*Indian Med. Gaz.*, Vol. LVIII, No. 3, p. 104.

— (1923*b*).—The incidence of kala-azar in Bengal.—*Indian Med. Gaz.*, Vol. LVIII, No. 7, p. 299.

(1923*c*).—The failure of 'Bayer 205' in the treatment of kala-azar.—*Indian Med. Gaz.*, Vol. LVIII, No. 9, p. 415.

— (1923*d*).—The treatment of kala-azar by meta-chlor-para-acetyl-amino-phenyl stibiate of sodium (von Heyden 471), 11 cases.—*Indian Med. Gaz.*, Vol. LVIII, No. 12, p. 578.

— (1924).—Report of the Kala-azar Research Department.—*Ann. Rep. Calcutta Sch. Trop. Med. and Hyg.* for 1923, p. 24.

— (1924*a*).—The reaction of the blood in kala-azar.—*Indian Journ. Med. Res.*, Vol. XI, No. 3, p. 719.

— (1924*b*).—The problem of cure in kala-azar.—*Indian Med. Gaz.*, Vol. LIX, No. 10, p. 492.

— (1925).—Report of the Kala-azar Research Department.—*Ann. Rep. Calcutta Sch. Trop. Med. and Hyg.* for 1924, p. 18.

NAPIER, L. EVERARD (1925*a*).—A preliminary note on the treatment of kala-azar by stibamine glucoside.—*Indian Med. Gaz.*, Vol. LX, No. 1, p. 24.

— (1925*b*).—A comparative study of the environment associated with kala-azar prevalence in Calcutta.—*Indian Journ. Med. Res.*, Vol. XII, No. 4, p. 755.

— (1925*c*).—A new organic antimony compound for the treatment of kala-azar.—*Indian Med. Gaz.*, Vol. LX, No. 12, p. 571.

— (1926).—Report of the Kala-azar Research Department.—*Ann. Rep. Calcutta Sch. Trop. Med. and Hyg.* for 1925, p. 21.

— (1926*a*).—An epidemiological consideration of the transmission of kala-azar in India.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 219.

— (1926*b*).—The pentavalent compounds of antimony in the treatment of kala-azar. I. Stibosan.—*Indian Journ. Med. Res.*, Vol. XIV, No. 2, p. 263.

— (1926*c*).—Reactions following the administration of the pentavalent compounds of antimony.—*Indian Med. Gaz.*, Vol. LXI, No. 11, p. 559.

— (1927).—The pentavalent compounds of antimony in the treatment of kala-azar. II. No. 693 (von Heyden).—*Indian Journ. Med. Res.*, Vol. XV, No. 1, in press.

NAPIER, L. EVERARD and DAS GUPTA, B. M. (1923).—Cultural examination of the urine in kala-azar.—*Indian Med. Gaz.*, Vol. LVIII, No. 11, p. 530.

NAPIER, L. EVERARD and FOSTER, P. (1927).—The control of kala-azar on tea estates.—*Indian Med. Gaz.*, Vol. LXII, No. 2, p. 76.

NAPIER, L. EVERARD and MUIR E. (1923).—*Kala-azar.*—Oxford Univ. Press, Calcutta.

NAPIER, L. EVERARD and SMITH, R. O. A. (1926.)—Further observations on the feeding of sandflies, *Phlebotomus argentipes*, on cases of kala-azar in Calcutta.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 147.

— (1926*a*).—A study of the bionomics of *Phlebotomus argentipes* with special reference to the conditions in Calcutta.—*Indian Journ. Med. Res. Memoirs*, No. 4, p. 161.

— (1927).—Development of *Leishmania donovani* in the gut of the sandfly, *Phlebotomus papatasii*.—*Indian Journ. Med. Res.*, Vol. XIV, No. 3, p. 713.

NICOLLE, C. (1925).—Chronique du Kala-azar en Tunisie.—*Archiv. l'Instit. Past. de Tunis*, Vol. XIV, No. 1, p. 136.

NOGUCHI, H. (1924).—Action of certain biological, chemical and physical agents upon cultures of leishmania; some observations on plant and insect herpetomonads.—*Proc. Internat. Conf. on Health Problems in Trop. America*, United Fruit Co., Boston, p. 455.

PATTON, S. W. (1907).—Development of Leishman-Donovan parasites in *Cimex rotundatus*.—*Scientific Memoirs*, No. 31, Government Printing, Calcutta.

— (1912).—Development of the parasite of Indian kala-azar.—*Scientific Memoirs*, No. 53, Government Printing, Calcutta.

PATTON, S. W. (1914).—The behaviour of the parasite of Indian kala-azar in the dog flea.—*Indian Journ. Med. Res.*, Vol. II, No. 1, p. 399.

— (1914*a*).—Examination of the peripheral blood of 84 patients suffering from kala-azar.—*Indian Journ. Med. Res.*, Vol. II, No. 2, p. 492.

— (1922).—Reflexions on the kala-azar and oriental sore problems.—*Indian Journ. Med. Res.*, Vol. IX, No. 3, p. 496.

PATTON, W. S. and HINDLE, EDW. (1926).—Notes on the species of sandflies (Genus Phlebotomus) of North China.—*Proc. Roy. Soc., Series B*, Vol. C, No. 704, p. 405.

PATTON, W. S., LA FRENAIS, H. M. and SUNDER RAO S. (1921).—Development of *L. tropica* in the bed-bug.—*Indian Journ. Med. Res.*, Vol. IX, No. 2, p. 240.

PIANESE, G. (1905).—Sull' anemia splenica infantile.—*Gazz. Internat. di Medicina*, Ann. VIII.

PITTALUGA, G. (1925).—Leishmaniose en Espagne.—*Publication of the League of Nations*, C.H. 368, Geneva.

RAY, C. B. (1921)—Hæmolytic test in kala-azar.—*Indian Med. Gaz.*, Vol. LVI, No. 1, p. 9.

— (1924)—The globulin content of serum in kala-azar.—*Indian Med. Gaz.*, Vol. LIX, No. 8, p. 387.

ROGERS, LEONARD (1897).—*Report on Kala-Azar.*—Assam Secretariat Printing Office.

— (1899).—The result of segregation of cases and moving from infected sites in eradicating the Assam epidemic malarial fever or kala-azar.—*Med.-Chir. Trans.*, Vol, LXXXII.

— (1904).—Preliminary note on the development of trypanosoma in cultures of the Cunningham-Leishman-Donovan parasites of cachexial fevers and kala-azar.—*Lancet*, Vol. CLXVII, p. 215.

— (1910).—*Fevers in the Tropics*, 2nd Edition.—Oxford Univ. Press, London.

— (1915).—Tartar emetic in kala-azar.—*British Med. Journ.*, Vol. II, p. 197.

— (1919).—Colloidal antimony sulphide intravenously in kala-azar —*Lancet*, Vol. CXCVI, p. 505.

ROGERS, L. and SHORTEN, A. J. (1915).—The alkalinity of the blood in kala-azar and cholera.—*Indian Journ. Med. Res.*, Vol. II, No. 4, p. 867.

ROSS, RONALD (1899).—Infectiousness of malarial fever and kala-azar.—*Indian Med. Gaz.*, Vol. XXXIV, No. 7.

ROW, R. (1922).—On the reversion of the flagellate form of *L. donovani* and *L. tropica* to the resistant non-flagellate torpedo and O-body in culture tubes.—*Indian Journ. Med. Res.*, Vol. X, No. 2, p. 23.

SHANKS, G. and KHAN, G. P. (1926).—The diagnosis of typhoid-like fevers, with special reference to typhoid and kala-azar.—*Indian Med. Gaz.*, Vol. LXI, No. 7, p. 324.

SHORTT, H. E. (1923).—*Herpetomonas ctenocephali* Fanthom ; some observations on its life history and reactions to different environments.—*Indian Journ. Med. Res.*, Vol. X, No. 3, p. 733.

— (1923*a*).—The infectivity of insect flagellates to vertebrates, with special reference to *H. ctenocephali.*—*Indian Journ. Med. Res.*, Vol. X, No. 4, p. 908.

— (1923*b*).—Record of kala-azar research work carried out at Shillong during 1922.—*Indian Journ. Med. Res.*, Vol. X, No. 4, p. 1150.

— (1923*c*)—Pathology of acute experimental kala-azar in monkeys.—*Indian Journ. Med. Res.*, Vol. XI, No. 1, p. 186.

— (1923*d*).—Recovery of *Herpetomonas donovani* from the urine of a kala-azar patient.—*Indian Journ. Med. Res.*, Vol. XI, No. 1, p. 319.

SHORTT, H. E. and BRAHMACHARI, U. N. (1925).—The chemotherapy of antimonial compounds in kala-azar ; part XIII.—*Indian Journ. Med. Res.*, Vol. XII, No. 3, p. 463.

SHORTT, H. E. and SEN, R. T. (1923).—Clinical kala-azar work performed in the special kala-azar hospital at Shillong during 1922.—*Indian Med. Gaz.*, Vol. LVIII, No. 7, p. 289.

SHORTT, H. E. and SWAMINATH, C. S. (1924).—A note on the infection of a mouse by means of bed-bugs, *Cimex hemiptera* Fabr. fed on the peripheral blood of a case of kala-azar.—*Indian Journ Med. Res.*, Vol. XI, No. 3, p. 965.

— (1925).—Experiments to decide whether the bed-bug, *C hemiptera* Fabr., can transmit Indian kala-azar.—*Indian Journ. Med. Res.*, Vol. XIII, No. 1, p. 143.

— (1925*a*).—Systemic infection of a monkey (*Macacus rhesus*) by intradermal inoculation of spleen puncture material from a case of Indian kala-azar.—*Indian Journ. Med. Res.*, Vol. XIII, No. 1, p. 149.

SIA, R. H. P. (1921).—Ray's hæmolytic test in kala-azar.—*China Med. Journ.*, Vol, XXXV, No. 5, p. 188.

— (1924).—A simple method of estimating quantitative differences in the globulin precipitation test in kala-azar.—*China Med. Journ.*, Vol. XXXVIII, No. 1, p. 35.

DA SILVA, PEREIRA (1913).—Notes sur le kala-azar.—*Arquivos do Inst. Bacteriol. Camara Pestana*, Vol. IV, No. 2, p. 147.

SINTON, J. A (1924).—Notes on some Indian species of the genus Phlebotomus. Part VIII.—*Indian Journ. Med. Res.*, Vol. XI, No. 4, p. 1035.

— (1925)—Notes on some Indian species of the genus Phlebotomus ; Part XII.—*Indian Journ. Med. Res.*, Vol. XII, No. 4, p. 789.

SMYLY, H. J. (1927).—Private communication.

SPAGNOLIO, G. (1920).—La cura del kalazar infantile con i preparati di antimonio.—*Giorn. di Clin. Med., Bologna*, Vol. I, No. 5, p. 182.

STEPHEN, A. (1897).—Dr. Rogers' report on kala-azar.—*Indian Med. Gaz.*, Vol. XXXII, No. 11, p. 408.

STEWART, A. D. (1926).—*Bengal Public Health Report for the Year 1924.*—Bengal Secretariat Book Depot, Calcutta.

THOMSON, D. S. B. (1911).—Report of the Kala-azar Commission to investigate the prevalence and cause of this disease in the Eastern Sudan.—*Fourth Report of the Wellcome Research Laboratories*, Vol. A, p. 143.

TURKUD, D. A., KRISHNAN, K. V. and IYER, P. V. S. (1926).—An investigation of kala-azar in certain endemic areas in Southern India.—*Indian Journ. Med. Res.*, Vol. XIII, No. 3, p. 703.

TWINING (1835).—*Clinical illustrations of the More Important Diseases of Bengal*, 2nd edition.

VIANNA and MARCHADO (1913).—Reports of two cases of muco-cutaneous leishmaniasis treated with intravenous tartar emetic.—*Bol. da Soc. Brazileira de Dermatologia*, Vol. II, No. 1, p. 17.

WATERSON, J. (1921).—A contribution to the knowledge of the bionomics of sandflies.—*Ann. Trop. Med. and Parasit.*, Vol. XVI, No. 1, p. 69.

WENYON, C. M. (1911).—Notes on the occurrence of herpetomonas in the phlebotomus of Aleppo.—*Journ. London Sch. Trop. Med.*, Vol. I, p. 98.

WENYON, C. M. (1911*a*).—Oriental sore in Bagdad ; together with observations on a gregarine of *Stegomyia fasciata*, a hæmogregarine of dogs and the flagellates of house-flies.—*Parasitology*, Vol. IV, p. 273.

— (1912).—Experiments on the behaviour of leishmania and allied flagellates in bugs and fleas, with some remarks on previous work.—*Journ. London Sch. Trop. Med.*, Vol. II, p. 13.

WRIGHT, J. H. (1903).—Protozoa in a case of tropical ulcer (Delhi boil).—*Journ. Med. Res.*, Vol. X, No. 3, p. 472.

YOUNG, T. C. MCCOMBIE (1924).—*Kala-azar in Assam.*—H. K. Lewis & Co., London.

YOUNG, C. W. (1923).—Kala-azar in China.—*China Med. Journ.*, Vol. XXXVIII, No. 10, p. 797.

YOUNG, C. W. and HERTIG, M. (1926).—Development of flagellates in Chinese sandflies fed on hamsters infected with *Leishmania donovani.*—*Proc. Soc. Exp. Biol. and Med.*, Vol. XXIII, No. 7, p. 611.

YOUNG, C. W., SMYLY, H. J. and BROWN, C. (1924).—Experimental kala-azar in the hamster.—*Proc. Soc. Exp. Biol. and Med.*, Vol. XXI, p. 357.

YOUNG, C. W. and VAN SANT, HELEN (1923).—*L. donovani* in the peripheral blood.—*Journ Exp. Med.*, Vol. XXXVIII, No. 3, p. 233.

YOUNG, T. C. MCCOMBIE (1927).—Some observations on sandflies in Bombay City.—*Indian Journ Med. Res.*, Vol. XIV, No. 3, p. 679.

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